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PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

VII. FERMENTATION OF WHEAT BY *AEROBACILLUS POLYMYXA* UNDER AEROBIC AND ANAEROBIC CONDITIONS¹

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Abstract

Aeration by mechanical agitation of 15% wheat mash fermented by *Aerobacillus polymyxa* inhibited the formation of 2,3-butanediol and particularly of ethanol. Aeration of similar mashes by passage of finely dispersed air or oxygen at the rate of 333 ml. per minute per litre of mash increased the rate of formation and yield of 2,3-butanediol but inhibited ethanol formation. However, the over-all time required for the completion of fermentation was not shortened from the usual 72 to 96 hr. required for unaerated mashes. There was no evidence of a shift from fermentative to oxidative dissimilation. Under aerobic conditions, the final butanediol-ethanol ratio was approximately 3 : 1. Anaerobic conditions, as produced by the passage of nitrogen or hydrogen through the mash, increased the rate of formation of both butanediol and ethanol and shortened the fermentation time to about 48 hr. Under these conditions, the butanediol-ethanol ratio was reduced to about 1.3 : 1.0. Carbon dioxide gave a butanediol-ethanol ratio resembling that of anaerobic fermentation but did not reduce fermentation time.

Introduction

Fermentation of carbohydrates by *Aerobacillus polymyxa* yields 2,3-butanediol and ethanol as the main products in a ratio of approximately 1.7 : 1.0 by weight. Since diol is the more valuable product it is desirable to obtain as high a proportion of it as possible without decreasing the total products. It is well known that vigorous aeration of *Aerobacter aerogenes* cultures stimulates production of diol. Assuming that ethanol is formed by reduction of acetaldehyde its formation by *Aerobacillus polymyxa* should be inhibited by aeration if oxygen is capable of competing with acetaldehyde as a hydrogen acceptor.

A study by Stahly and Werkman (4) on the mechanism of acetoin formation by *A. polymyxa* showed that under aerobic conditions there was a fourfold increase in acetoin, no gain in diol, and a marked decrease in ethanol as compared with anaerobic conditions. Adams and Stanier (2) have reported the results of comparative fermentations of glucose by *A. polymyxa* in atmospheres of air and nitrogen. Ethanol formation was depressed but no

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appreciable increase in acetoin was found under aerobic conditions. Analyses performed at 10-hr. intervals showed a slightly slower fermentation in air.

The experiments reported in the present paper were undertaken to provide more information on the effects of aerobic and anaerobic conditions on the fermentation of wheat by *A. polymyxa*. In particular, means were sought for attaining a higher diol-ethanol ratio.

Wheat mashes of a concentration normally used in commercial fermentation processes instead of dilute sugar solutions were fermented in all experiments involving *A. polymyxa*. This medium permitted study of such factors as aeration, mixing, and foaming, as well as fermentation efficiency of the organism on a complex and concentrated substrate. In view of the results obtained with *Aerobacillus* fermentations, similar experiments involving air and nitrogen treatments were conducted with another diol-producing organism, *Aerobacter aerogenes*.

Materials and Methods

Fifteen per cent wheat mashes containing 1% of calcium carbonate served as fermentation media. In all experiments described in this paper two strains of *A. polymyxa*, N.R.C. C4 (2) and C3 (2), respectively, from our own collection, were used. The mashes were inoculated with a culture of the organism, brought up in two 24-hr. stages on whole-wheat-yeast-extract-calcium-carbonate media. An amount of inoculum equal to 3% of the volume of the mash was found to be sufficient. All mashes were fermented at 30° C. The methods of analysis for butanediol, ethanol, acetoin, and organic acids have been previously described (5).

Aeration by Agitation

A simple and effective method of aerating bacterial cultures during fermentation is by means of agitation on a mechanical shaker. Since this procedure has been used with good results on *Aerobacter aerogenes**, it was chosen as a means of aerating *Aerobacillus polymyxa*.

Continuous Agitation

Four hundred millilitres of 15% whole wheat mash in 2000-ml. flasks was agitated on a mechanical shaker throughout the experiment. The shaker has a total linear excursion of 2 in. at the rate of 180 cycles per minute. The mashes were inoculated with the two strains, each in duplicate, and for all experiments unshaken controls were included. Duplicate sets of flasks were removed from the shaker at 72 and 96 hr., respectively, and along with their unshaken controls were analysed for diol and ethanol.

It is apparent from the average results in Table I that aeration produced by agitation inhibited the formation of diol and ethanol by both organisms. The relatively higher diol-ethanol ratios in the agitated mashes show that the

* Johnson, M. J., Department of Biochemistry, University of Wisconsin. Private communication.

TABLE I

EFFECT OF AERATION BY AGITATION ON THE FERMENTATION OF 15% WHOLE WHEAT MASH BY STRAINS OF *A. polymyxa*

Treatment	Fermen- tation period, hr.	Butanediol, %		Ethanol, %		Total products, %		Ratio	
		N.R.C. C3 (2)	N.R.C. C4 (2)	N.R.C. C3 (2)	N.R.C. C4 (2)	N.R.C. C3 (2)	N.R.C. C4 (2)	N.R.C. C3 (2)	N.R.C. C4 (2)
None Agitation	72	2.66	2.50	1.46	1.24	4.12	3.74	1.82	2.01
	—	2.14	1.59	0.87	0.49	3.01	2.08	2.46	3.25
None Agitation	96	2.85	2.86	1.46	1.39	4.31	4.25	1.95	2.06
	—	2.80	2.30	1.26	0.67	4.06	2.97	2.22	3.43

formation of ethanol is depressed more than that of diol. While it is impossible to segregate the individual effects of mixing and aeration in this experiment, the inhibition of ethanol formation would seem to be due to aeration, since it has been shown that exposure of large surfaces of mash to air has the same effect (1). The response of the two organisms to agitation is different, C4 (2) being inhibited to a greater extent than C3 (2). The overall inhibitory effect on both organisms is less marked at 96 than at 72 hr., since the fermentations are then approaching completion.

Intermittent Agitation

Since prolonged agitation of the fermentation mash was not beneficial, it was decided to investigate the effect of agitation followed by a period of quiescence. It is well known that in yeast fermentations an initial aeration period increases the number of organisms, and it was thought that if a similar condition could be brought about in *A. polymyxa* fermentations the increased numbers would lead to a more rapid fermentation.

Mashes were shaken for 0, 12, 24, 36, 48, 60, 72, 84, and 96 hr. At the end of each agitation period the flasks were removed from the shaker and allowed to stand for the remainder of the fermentation time. Duplicate mashings were used and analyses for diol and ethanol made at 72 and 96 hr. The fermentation was carried out with *A. polymyxa* C4 (2).

The results of 72-hr. fermentations given in Table II show that any period of agitation is detrimental in so far as total yield of diol and ethanol is concerned. Increasing the agitation time caused a progressive rise in the diol-ethanol ratio and indicated that the inhibitory effect is more pronounced on ethanol than on diol formation. It is interesting to note that diol production was most markedly repressed by a shaking period of about 48 hr., while, on the other hand, ethanol was inhibited almost uniformly by any shaking time greater than 12 hr. After fermentation for 96 hr., the inhibitory effects were less marked, especially on diol yield, which was almost as high as that of the control. The ethanol was not much greater than at 72 hr. It appears that the mechanism of ethanol formation was permanently inhibited, while the diol mechanism became partially adapted to the conditions.

TABLE II

EFFECT OF INTERMITTENT AGITATION ON THE FERMENTATION OF 15%
WHOLE WHEAT MASH BY *A. polymyxa* N.R.C. C4 (2)

Treatments		Analyses at 72 hr.			
Agitation time, hr.	Standing time, hr.	Butanediol, %	Ethanol, %	Total products, %	Ratio
0	72	2.51	1.36	3.87	1.85
12	60	2.36	1.18	3.54	2.00
24	48	1.83	0.70	2.53	2.62
36	36	1.77	0.61	2.38	2.93
48	24	1.44	0.50	2.06	3.08
60	12	1.98	0.62	2.60	3.17
72	0	2.22	0.64	2.87	3.45
		Analyses at 96 hr.			
0	96	2.12	1.60	4.52	1.82
84	12	2.70	0.80	3.50	3.38
96	0	2.83	0.75	3.58	3.77
Standing time, hr.	Agitation time, hr.	Analyses at 72 hr.			
0	72	2.31	0.61	2.92	3.76
12	60	2.37	0.75	3.12	3.17
24	48	2.39	0.79	3.18	3.03
36	36	2.68	1.15	3.83	2.33
48	24	2.94	1.43	4.37	2.06
60	12	2.94	1.53	4.47	1.93
72	0	2.80	1.52	4.32	1.84
		Analyses at 96 hr.			
0	96	3.00	0.92	3.92	3.26
84	12	3.13	1.70	4.83	1.84
96	0	3.00	1.63	4.63	1.84

In a further experiment the conditions for agitation just described were reversed, and a standing period preceded that of shaking. Evidence has been presented in another paper to show that rapid removal of the gases increases the fermentation rate (1). It was thought that if agitation followed a period of standing during which the fermentation had become well started, release of the fermentation gases might increase the rate.

The mashes prepared by procedures already described were allowed to stand for 0, 12, 24, 36, 48, 60, 72, 84, and 96 hr., respectively, and at the end of these times were shaken for the remainder of the fermentation periods. Two fermentation periods, 72 and 96 hr., were used, and analyses for diol and ethanol were made at the end of each of these times. All mashes were duplicated.

The results of the experiment are given in Table II. Agitation of the mash produced a marked inhibition on ethanol and lesser effect on diol formation. There is, however, a slightly beneficial effect on diol formation in mash shaken for the last 12 to 24 hr. of a 72-hr. fermentation period. The results of the 96-hr. fermentations show a similar effect, which extends to ethanol formation as well.

Aeration with Various Gases

Although the previous experiments involving agitation of mash showed inhibition of the *A. polymyxa* fermentation, they also demonstrated that aerobic conditions altered the diol-ethanol ratios in favour of the former. It seemed worth while, therefore, to make a more detailed study of this fermentation under aerobic, as well as anaerobic, conditions. For aerobiosis, oxygen and air were used; for anaerobiosis, nitrogen, hydrogen, and carbon dioxide.

These gases were bubbled through 1500-ml. portions of inoculated 15% wheat mash in 3500-ml. Kluver flasks, which were fitted with "medium" sintered glass disks for gas dispersion. The compressed gases were passed through the mash at a rate of 333 ml. per minute per litre of mash. Controls consisted of mash fermented under similar conditions, but without aeration or agitation. The vapours from the mash were condensed at -70°C . and the condensates added back before sampling for analysis. The samples were withdrawn under sterile conditions at 24, 48, 72, 96, and 120 hr., and analysed for diol, ethanol, acetoin, and, in some cases, total organic acids. The analytical results for each set of conditions are given in Tables III, V, and VI. All data represent means of four separate fermentations.

In general, the method of aeration provided thorough mixing and distribution of the gas. During the first 12 hr. of fermentation the mash was fairly thick and viscous, and the dispersion of gas was not satisfactory, especially toward the edge of the flask. However, at 24 hr., liquefaction of the mash had reached a stage that permitted distribution of fine bubbles throughout the entire mash. No foaming or frothing difficulties were encountered in the apparatus used.

In Table III yields of diol by C4 (2) and C3 (2) are given. The control for C4 (2) indicates a typical fermentation with the maximum rate occurring in the 24 to 48 hr. period, levelling off at about 72 hr., and proceeding slowly to completion in approximately 120 hr. Under aerobic conditions brought about by oxygen, there was a marked increase in the rate of formation of diol, and the final yield was 13% greater than that of the controls. The air-treated mash did not have the same accelerated rate of diol formation, although final yields were equal to those of oxygenated mash. Nitrogen (anaerobic conditions) increased the rate of diol formation more than any other gas but the final yield was less than that of the control. Hydrogen, which is a normal product of the fermentation, increased the rate in the

early stages as compared with that of the control, but not to the same extent as nitrogen. However, final yield of diol was the same at 120 hr. Carbon dioxide, the main gaseous product, increased diol formation only slightly as compared with that of the control, and the final yield was the same as in other anaerobic gases. The results for C3 (2) agreed in general with those of C4 (2), the main difference being the more rapid fermentation rate of the former.

The passage of a vigorous stream of gas through a fermentation mash provides mechanical agitation and facilitates the escape of the fermentation gases. It has already been claimed that the fermentation rate is increased by conditions that provide for rapid removal of carbon dioxide (1). The fact that aeration with carbon dioxide had little effect on the rate lends support to this claim. On the other hand, gases capable of removing carbon dioxide from the mash, e.g., nitrogen, hydrogen, oxygen, and air, all give increased rates of diol formation.

The effects of aerobic and anaerobic conditions on ethanol production are given in Table III also. The control for C4 (2) showed that ethanol

TABLE III
FERMENTATION OF 15% WHOLE WHEAT MASH UNDER AEROBIC AND ANAEROBIC CONDITIONS BY *A. polymyxa*

Organism	Aeration treatment	Butanediol, %					Ethanol, %				
		Time, hr.					Time, hr.				
		24	48	72	96	120	24	48	72	96	120
C3 (2)	Control	0.76	1.85	2.44	2.60	2.70	0.48	1.13	1.55	1.68	1.69
	Air	0.86	1.91	2.68	2.91	2.88	0.44	0.86	1.25	1.33	1.31
	O ₂	1.13	2.73	3.06	3.04	3.04	0.42	0.95	1.08	1.10	1.09
	N ₂	1.31	2.33	2.44	2.47	2.48	0.86	1.82	1.88	1.87	1.85
	CO ₂	0.65	1.93	2.40	2.52	2.59	0.45	1.23	1.73	1.90	1.81
	H ₂	0.93	1.99	2.43	2.59	2.62	0.65	1.71	1.86	1.91	1.91
C4 (2)	Control	0.67	1.47	2.18	2.51	2.71	0.40	0.81	1.23	1.51	1.65
	Air	0.61	1.36	2.28	2.90	3.09	0.30	0.55	0.86	1.05	0.98
	O ₂	1.02	2.03	2.88	2.95	3.05	0.36	0.56	0.80	0.87	0.94
	N ₂	1.10	2.47	2.51	2.49	2.51	0.73	1.76	1.88	1.85	1.83
	CO ₂	0.70	1.62	2.35	2.46	2.51	0.45	1.00	1.46	1.62	1.61
	H ₂	0.91	2.13	2.51	2.54	2.54	0.56	1.44	1.78	1.90	1.90

formation in a normal fermentation was complete in 120 hr. The effect of aerobic conditions was to decrease the yield of ethanol markedly, the production yields being only slightly over half that of the control. On the other hand, anaerobic conditions as brought about by nitrogen and hydrogen gases caused a marked increase in rate of formation as well as final yield of ethanol. The initial rate with hydrogen is less than that with nitrogen, but final yields were substantially the same. Carbon dioxide caused only a slightly increased rate compared with that of the control. The results with C3 (2) followed,

in general, the pattern of those of C4 (2); the faster over-all rate of fermentation by C3 (2) was again apparent. It is clear from these observations that ethanol formation is retarded by the presence of a hydrogen acceptor (oxygen).

In Table IV the diol-ethanol ratios are given and show more clearly the relation between the products formed during fermentation. The ratio for

TABLE IV

EFFECT OF AEROBIC AND ANAEROBIC CONDITIONS ON THE RELATIVE AMOUNTS OF DIOL AND ETHANOL FORMED IN WHOLE WHEAT FERMENTATION BY *A. polymyxa*

Organism	Aeration treatment	Diol-ethanol ratio				
		Time, hr.				
		24	48	72	96	120
C3 (2)	Control	1.58	1.64	1.57	1.55	1.60
	Air	1.95	2.22	2.14	2.19	2.20
	O ₂	2.69	2.87	2.83	2.76	2.79
	N ₂	1.52	1.28	1.30	1.32	1.34
	CO ₂	1.44	1.57	1.39	1.40	1.43
	H ₂	1.43	1.16	1.30	1.35	1.37
C4 (2)	Control	1.67	1.81	1.77	1.66	1.64
	Air	2.03	2.47	2.65	2.76	3.15
	O ₂	2.83	3.62	3.60	3.39	3.25
	N ₂	1.51	1.40	1.33	1.35	1.37
	CO ₂	1.56	1.62	1.61	1.52	1.56
	H ₂	1.62	1.48	1.41	1.34	1.34

the control experiment using C4 (2) was fairly consistent, but under aerobic conditions was greatly increased. Its progressive increase with time showed that the production of ethanol is greatly inhibited, while that of diol is actually stimulated. The decrease in ratio in the C4 (2) fermentation at 96 and 120 hr. under nitrogen is explained by a slow increase in the amount of ethanol after that of the diol has become constant. Under anaerobic conditions the ratio, which was approximately 1.5 : 1.0 at 24 hr., dropped to a level of about 1.3 : 1.0 during the 48 to 72 hr. period, when the fermentations were relatively complete, and remained fairly constant thereafter. The ratio in the carbon dioxide treated mashes is intermediate between those of the controls and the anaerobic mashes in the late stages of fermentation.

The effects of the various treatments on acetoin production are shown in Table V. In the controls there is a continual increase to 96 hr., although the amount is not large. Under aerobic conditions there is a marked increase, especially with oxygen. It is interesting to note that the amount is quite small in comparison with that known to occur under similar conditions with *Aerobacter aerogenes* cultures. The fact that the increase in acetoin is not accompanied by a corresponding decrease in diol indicates that it is not being formed by diol oxidation. The accumulation of acetoin is probably due to retardation of its reduction to diol in the presence of excess oxygen.

The amount formed under anaerobic conditions is comparable to that in the control, and tends to become constant after 72 hr.

The 'total products' data recorded in Table V are the sums of diol, ethanol, and acetoin. Organic acid was not included, since it was determined only in certain experiments. Reference to Table VII shows, however, that a

TABLE V
FERMENTATION OF 15% WHOLE WHEAT MASH UNDER AEROBIC AND ANAEROBIC CONDITIONS BY *A. polymyxa*

Organism	Aeration treatment	Acetoin, %					Total products, %				
		Time, hr.					Time, hr.				
		24	48	72	96	120	24	48	72	96	120
C3 (2)	Control	0.03	0.09	0.15	0.14	0.15	1.27	3.07	4.14	4.42	4.54
	Air	0.05	0.15	0.17	0.21	0.29	1.35	2.92	4.10	4.45	4.48
	O ₂	0.06	0.12	0.26	0.38	0.41	1.61	3.80	4.40	4.52	4.54
	N ₂	0.07	0.16	0.12	0.11	0.12	2.24	4.31	4.42	4.45	4.45
	CO ₂	0.03	0.09	0.09	0.11	0.08	1.13	3.25	4.22	4.43	4.48
	H ₂	0.04	0.10	0.08	0.08	0.06	1.62	3.80	4.37	4.58	4.59
C4 (2)	Control	0.04	0.05	0.07	0.12	0.12	1.11	2.33	3.48	4.14	4.48
	Air	0.04	0.06	0.09	0.16	0.22	0.95	1.97	3.23	4.11	4.29
	O ₂	0.05	0.10	0.22	0.40	0.50	1.43	2.69	3.90	4.22	4.49
	N ₂	0.05	0.10	0.12	0.13	0.13	1.88	4.33	4.51	4.47	4.47
	CO ₂	0.04	0.07	0.09	0.12	0.10	1.19	2.69	3.90	4.20	4.22
	H ₂	0.04	0.07	0.10	0.10	0.10	1.51	3.64	4.39	4.54	4.54

theoretical yield of fermentation products was obtained when the organic acids were included. The 'total products' as calculated in Table V serve as an indication of the over-all rate of the fermentations, as well as the extent of their completeness. Under the conditions of the experiment the control fermentation for C4 (2) was complete in 120 hr., and the total product yield was 4.48%. Under aerobic conditions produced by oxygen, fermentation was complete in 120 hr., and the rate was only slightly greater than that of the control. Air treated mashes also were complete in 120 hr. As the previous discussion on diol-ethanol ratios showed, aerobic conditions increased the rate of formation of diol and inhibited that of ethanol. Anaerobic conditions brought about by nitrogen and hydrogen increased the rates of formation of both diol and ethanol, and significantly shortened the times required for complete fermentation. Carbon dioxide accelerated the rate for the first 48 hr., but the fermentation did not attain completeness in 120 hr.

The concordance of the results for two strains indicates that the effects of aerobic and anaerobic conditions as demonstrated here are common to all *A. polymyxa* strains.

Table VI reports the total organic acid contents of mashes fermented under aerobic and anaerobic conditions by C3 (2) and C4 (2). The main point of interest in comparison with the controls is the marked increase in acid produc-

TABLE VI

ORGANIC ACID PRODUCTION IN AEROBIC AND ANAEROBIC FERMENTATIONS OF WHOLE WHEAT MASH BY *A. polymyxa* AT 120 HR.

Organism	Control		Oxygen		Air		Nitrogen	
	pH	Organic* acids	pH	Organic acids	pH	Organic acids	pH	Organic acids
C4 (2)	5.63	33.60	5.47	90.10	5.49	88.60	5.84	61.60
C3 (2)	5.72	43.70	5.60	85.00	5.58	78.80	5.90	60.04

* Total organic acids are calculated as cc. N/10 acid per 100 gm. of mash.

tion under aerobic conditions and a smaller increase under anaerobic conditions. Under anaerobic conditions a higher pH is associated with a relatively high acid content. This suggests that some proteolysis may have taken place and that ammoniacal products were formed that reacted with the acid. This hypothesis is given some support by the observation that the pH of fermented mashes rises on long standing. Furthermore, ammonia has been found in wheat mashes fermented by *A. polymyxa* (1).

The data from the analyses for diol, acetoin, ethanol, and organic acids were recalculated as millimoles of each product formed per 100 millimoles of glucose fermented, and are given in Table VII. In the same table the "glucose

TABLE VII

EFFECT OF AEROBIC AND ANAEROBIC CONDITIONS ON THE DISTRIBUTION AND YIELD OF PRODUCTS IN FERMENTATIONS OF A 15% WHOLE WHEAT MASH BY *A. polymyxa* N.R.C. C3 (2)

* Glucose fermented: 53.2 millimoles per 100 ml.

Product	Millimoles of product per 100 millimoles of glucose fermented			
	Control	Air	Oxygen	Nitrogen
2,3-Butanediol	56.5	60.1	63.5	51.9
Acetoin	3.2	6.2	8.6	2.6
Ethanol	69.1	50.7	44.5	75.2
Organic acids	8.1	14.8	16.0	11.3
Glucose accounted for, %	98.3	99.0	102.3	97.8

* Glucose calculated on the basis of complete fermentation of a 15% wheat mash; original wheat contained 55% of starch and 3.5% of sugar.

accounted for" is calculated by assuming that one mole of glucose is required to produce each of the following: 1 mole of diol, 1 mole of acetoin or 2 moles of either ethanol or acid. Since the results show that the "glucose accounted for" always approximated 100%, it can be concluded that the fermentations

were complete, and that the main products had been determined. The data also confirm the observations previously made that under aerobic conditions there were proportional increases in yields of diol and acetoin and a diminution in yield of ethanol as compared with anaerobic conditions.

Fermentation of Glucose by *Aerobacter aerogenes* under Aerobic and Anaerobic Conditions

Since it has been shown that the fermentation rate of *Aerobacillus polymyxa* was markedly increased under anaerobic conditions, it seemed worth while to examine another diol producing organism, *Aerobacter aerogenes*, under similar conditions. As pointed out earlier, it is well known that vigorous aeration of *Aerobacter* cultures is needed to bring about rapid fermentation with a high proportion of diol. From the *Aerobacillus* results it seemed possible that the rate of *Aerobacter* fermentations should be increased under anaerobic conditions, but the diol-ethanol ratio would be much lower.

An experiment was set up to explore these possibilities. The culture medium consisted of 10% of dextrose, 0.09% of potassium monohydrogen phosphate, 0.09% of potassium dihydrogen phosphate, 0.025% of magnesium sulphate, and 0.2% of urea. Six-hundred-millilitre portions of the sterilized media were put in each of three Kluver flasks, and inoculated with 9 ml. of a 24-hr. culture of *Aerobacter aerogenes*, N.R.R.L No. 199. Air was passed into one flask at the rate of 1 ml. per min. per ml. of mash, nitrogen into another at the same rate, and the third served as an unaerated control. Analyses for diol and ethanol were made at the end of 24 hr. (Table VIII). The fermenta-

TABLE VIII

EFFECT OF AEROBIC AND ANAEROBIC CONDITIONS ON THE FERMENTATION OF GLUCOSE BY *Aerobacter aerogenes* N.R.R.L 199

Aeration treatments	Butanediol, %	Ethanol, %	Total products, %	Ratio
None	0.31	0.21	0.52	1.47
Air	3.01	0.89	3.90	3.35
Nitrogen	2.57	1.47	4.04	1.75

tion rate was greatly increased by the passage of air or nitrogen through the medium. The variation in the diol-ethanol ratio indicated that under vigorous aeration the rate of formation of diol is increased, that of ethanol being depressed; under nitrogen the formation of diol is not accelerated to the same extent, but that of ethanol is greatly increased. The yield of almost equal amounts of total products in 24 hr. showed that the over-all rate of fermentation is approximately the same under aerobic and anaerobic conditions.

A comparison of these results with those obtained with *Aerobacillus* shows a marked similarity in the response of the two organisms to aerobic and anaerobic conditions. The over-all rate of fermentation is a composite of the rates of formation of diol and ethanol, respectively, and is indicated by the time required to reach maximum yields of the two products. In comparison with the controls, the rate of formation of both diol and ethanol is increased under anaerobic conditions by *Aerobacillus* and *Aerobacter* and hence fermentation time is decreased. Under aerobic conditions, diol production by both organisms is stimulated (although to a greater extent by *Aerobacter*), while ethanol formation is markedly inhibited. As a result, in an atmosphere of air or oxygen, fermentation time for *Aerobacter* is greatly decreased while that of *Aerobacillus* is relatively unaffected.

During the preparation of this paper, the author's attention was drawn to a patent issued to Scheffer (3) in which it is claimed that aeration with hydrogen or nitrogen of mash fermented by bacteria capable of producing 2,3-butylene glycol (2,3-butanediol) increases the fermentation rate. An example with *Aerobacter aerogenes* is given in which fermentation gases freed of carbon dioxide were successfully used for aeration purposes. The results reported in the present paper on the *Aerobacter* fermentation substantiate the aeration claim. The broad claim, unsupported by experimental evidence in the patent, that other bacteria capable of producing 2,3-butanediol would respond in the same manner has been substantiated also by our results with *Aerobacillus polymyxa*.

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PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

VIII. pH CONTROL IN *AEROBACILLUS POLYMYXA* FERMENTATIONS AND ITS EFFECTS ON PRODUCTS AND THEIR RECOVERY¹

BY G. A. ADAMS² AND J. D. LESLIE³

Abstract

Comparative studies have shown that the pH of 15% wheat mashes fermented by *Aerobacillus polymyxa* can be as satisfactorily controlled by ammonium hydroxide as by calcium carbonate. The formation of 2,3-butanediol and ethanol was unaffected by all pH levels tested (5.8, 6.0, 6.5, 7.0) with the possible exception of pH 7.0, where a slight diminution of diol formation appeared at 96 hr. Over the pH range 5.8 to 6.0, the amount of ammonium hydroxide required, the escape of ammonia from the mash, and the production of acid were all minimized. The consumption of ammonia was greatest in the first 36 hr. of the fermentation owing to rapid acid production. Fermentation at the different pH levels did not affect the butanediol-ethanol ratio, which was approximately 1.5.

Replacement of calcium carbonate by ammonium hydroxide reduced the ash content of the unfermented residue from approximately 20 to 4%. Protein contents ($N \times 5.7$) of insoluble residues from carbonate and ammonia treated washes were 25 and 32%, respectively. In both mashes approximately 50% of the unfermented solids were soluble.

Calculation of carbon balances on fermentables showed that increased acid production was accompanied by a decrease in carbon dioxide formation.

Riboflavin and nicotinic acid contents per 100 gm. of fermented mash averaged 19.2 and 1270 $\mu\text{gm.}$, respectively and were unaffected by pH of fermenting mash and heat treatment at 100° C. for 10 hr. The riboflavin showed an 80% increase over that present in the original wheat; nicotinic acid showed a 40% decrease.

Introduction

Carbohydrate dissimilation by *Aerobacillus polymyxa* yields, in addition to diol and ethanol, small amounts of organic acids, which, if left unneutralized, cause the pH to drop to a level of about 5.0. At this level the fermentative process is almost completely inhibited. Previous experiments have shown that addition of 0.5 to 1.0% of calcium carbonate prevents the pH in this fermentation from falling below the range 5.6 to 5.8 (5). Although calcium carbonate is the usual agent for controlling acidity in bacterial fermentations there are several objections to its use in large scale operations with *A. polymyxa*. It is impossible to maintain a uniform pH at a desired level throughout the fermentation period, calcium carbonate increases the ash content in the unfermented residues thereby rendering them less desirable as stock feed, and cooking whole wheat mashes with carbonate at high temperatures has a deleterious effect on the subsequent fermentation. This can be avoided by sterilizing the carbonate separately but such a procedure involves extra equipment and labour.

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After consideration of other possible agents for controlling pH, ammonia was selected as the most suitable. It overcomes the objections outlined above in that it can be added as required throughout the course of the fermentation, thus permitting the maintenance of any desired pH level. Ammonia gas and its concentrated aqueous solution are both inherently sterile and hence may be introduced directly into fermentation media. From an industrial point of view, ammonia meets the requirements of a plentiful and relatively inexpensive chemical; in addition it can be handled conveniently either as a gas or in aqueous solution, with or without automatic control.

The main purposes of the present investigation were threefold: (1) to compare the relative merits of fermentations the pH levels of which were controlled with ammonia and calcium carbonate, respectively; (2) if ammonia was satisfactory to determine the optimum pH within the range 5.6 to 7.0; and (3) to compare the relative effects of the two agents on the nature, separation, and recovery of the products and by-products.

Since nitrogen is known to be a factor in the nutrition of the organism it might be anticipated that addition of ammonia would affect the fermentation reactions, e.g., rate, completeness, distribution of fermentation products, as well as the distribution of nitrogen in the soluble and insoluble fractions.

Methods and Materials

General

To attain the objectives of the investigation it was necessary to make quantitative measurements of total materials and main components at each step and to calculate material balances; from these balances, losses and distributions could be determined.

Approximately 15% whole wheat mash was prepared as previously described (5), and 6-litre lots were dispensed into 12-litre flasks. All mashes were inoculated, after cooling, with known amounts of an *A. polymyxa* strain, C3 (2), prepared on 5% whole wheat mash. The fermentation temperature was 30° C. throughout; fermentation times varied from 72 to 120 hr.

For pH control with calcium carbonate 1.0% of the powdered solid was sterilized separately and mixed with the cooked mash prior to inoculation. For control with ammonia the pH of the mash was adjusted at periodic intervals by one of the following methods.

Colorimetric: 10 ml. of alcoholic bromthymol blue was added to each mash, and the pH adjusted by addition, under sterile conditions, of a measured quantity of concentrated ammonium hydroxide (28% ammonia). A sterile sample of the mash containing the indicator and adjusted to pH 6.5 served as a colour reference standard.

Electrometric: A 50 ml. portion of mash was removed from each flask under sterile conditions, and the pH measured and adjusted to the desired level with dilute ammonium hydroxide of known strength. The calculated amount of concentrated ammonium hydroxide was then added to the mash.

In the recovery phases of the work two operations were carried out; filtration of the fermented mashes to separate the insoluble residues from the liquors, and distillation of either the mash or filtrate. Filtration was satisfactorily accomplished through several layers of cheesecloth in a box filter; the filtering area measured 16 by 24 in. Simple distillation of 1- to 2-litre batches was carried out in apparatus of the standard laboratory glassware type using a 3-litre flask and short adapter leading to the condenser; the upper part of the flask and the adapter were lagged to minimize reflux. Distillation was carried to the point where the residues showed definite signs of thermal decomposition, and the distillates were collected fractionally or *in toto* for pH measurement. Batch rectification was done in the same way except that an 18 in. Vigreux column was added to the apparatus. The column was lagged and reflux was supplied by an air jet at the top.

Analytical

The ground wheat used for mash preparation was analysed for moisture by the A.O.A.C. method (2), nitrogen by a Kjeldahl determination, starch by polarimetry (3), and reducing sugars according to Hanes (4). Solids in the unfermented mashes were measured by evaporating a 50 gm. aliquot and drying the residue to constant weight in an electric oven at 100° C.

The fermented mashes were analysed for diol and ethanol by methods previously described (5). In addition, total solids were measured by the same procedure as described above except that it was necessary to correct the weight of the dried solids for the diol remaining after drying. The latter was estimated in an aqueous extract of the dried solids.

Total acids in the fermented mashes were determined by ether extraction of a 100 ml. portion, at pH 2, for 24 hr. The solvent was removed, the acid extract made up to 100 ml. with water, and an aliquot titrated with *N*/10 sodium hydroxide. No determination of the individual acids was attempted and the results are reported as grams of acetic acid per 100 ml. of mash.

The filtrates were analysed for diol, ethanol, and total solids by the procedures outlined above for mashes; in addition, total nitrogen was determined by the Kjeldahl method. Ammoniacal nitrogen was estimated by making a sample alkaline and aspirating the free ammonia into standard acid.

The residues separated by filtration were thoroughly mixed and 500-gm. portions dried at 100° C. The dried residues, after being corrected for diol as before, were analysed for Kjeldahl and ammoniacal nitrogen, and also for ash by the A.O.A.C. method (2).

Riboflavin and nicotinic acid were estimated by microbiological methods*.

Experimental Results

The first experiments were designed primarily to test the feasibility of controlling a fermentation by addition of ammonia solution in comparison

* These analyses were kindly performed by W. A. Crandall, Laboratory of Hygiene, Department of National Health and Welfare, Ottawa.

with the usual method employing 1% calcium carbonate. From general knowledge of bacterial fermentations a pH of 6.5 was arbitrarily chosen for the ammonia controlled mashes.

In Expt. 1 the pH was adjusted at 12-hr. intervals by the colorimetric method. A serious drawback of this procedure was found to be a partial decolorization of the dye by some fermentation product, making pH adjustments unreliable. The fact, however, that the pH at the end of the experiment was approximately 6.6 (by electrometric measurement) indicated that reasonable control had been achieved.

To obtain closer pH control in Expt. 2 the electrometric method was adopted. Although this method permits more accurate control there is a marked drop in pH in the intervals between adjustments, especially during the early stages of the fermentation. The magnitude of these variations is shown in Fig. 1.

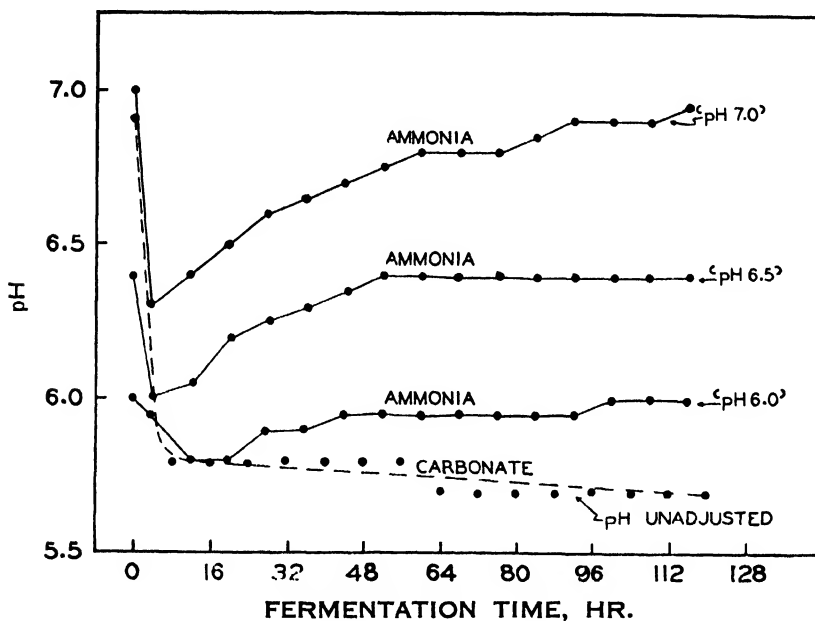


FIG. 1. Variation of average pH in fermentations controlled with ammonia and with calcium carbonate.

The fermentation data for the first two experiments are summarized in Table I.

Fermentation efficiencies have been calculated as the ratio of the actual yield of diol plus ethanol to the theoretical yield; the method of calculating the latter, considering starch as the only source of fermentables, has been previously described (5). The results show that in one experiment the efficiency was higher in the carbonate mash than in the ammonia mashes, but that in the other experiment the reverse was true. This leads to the con-

TABLE I

SUMMARY OF DATA ON *A. polymyxa* FERMENTATIONS USING AMMONIA AND CALCIUM CARBONATE FOR pH CONTROL

(Specific gravity of mash, 1.015)

	Expt. 1			Expt. 2		
	Controlled with:			Controlled with:		
	Ammonia		Carbonate†	Ammonia		Carbonate‡
Weight of original mash, gm.	6500	6555	6595	6335	6394	6223
Wheat in mash, %*	14.30	14.26	13.87	15.16	15.20	15.62
Inoculum, ml.	300	300	300	200	200	200
Ammonium hydroxide (28%) added, ml.	62.0	62.0	—	40.8	42.0	—
Corrected ammonium hydroxide, ml.**	62.0	62.0	—	42.0	43.3	—
Weight of ammonia added per kgm. 15% mash, gm.	2.52	2.51	—	1.65	1.68	—
Weight of mash after fermentation, gm.	6570	6615	6665	5820	5857	6038
Weight of samples removed during fermentation, gm.	0	0	0	406	406	0
Time of fermentation, hr.	74	74	74	93	93	93
Final pH of mash	6.71	6.55	5.60	6.50	6.50	5.70
Diol in fermented mash, %	2.33	2.27	2.41	2.78	2.64	2.64
Ethanol in fermented mash, %†	1.73	1.79	1.80	1.85	1.95	1.60
Diol-ethanol ratio	1.35	1.27	1.34	1.50	1.35	1.65
Fermentation efficiency, %	94.0	94.3	100.1	103.5	102.4	94.0
Total solids in fermented mash, %	4.46	4.40	5.63	4.99	4.92	6.45
Unfermented solids, %						
Original wheat	31.1	30.8	40.6	32.4	32.3	41.3
Theor. unfermented solids, %						
Original wheat	32.3	32.3	32.3	32.8	32.8	32.8

† Sixty gm. of carbonate included in weight of mash.

* Wheat analyses on "as is" basis: Expt. 1—Moisture, 13.0; starch, 54.7; nitrogen, 2.3%.

Expt. 2—Moisture, 13.6; starch, 53.6; nitrogen, 2.1%.

** Corrected for sampling.

† In Expt. 1, ethanol corrected for indicator solvent.

clusion that in spite of fortuitous variations there is no essential difference in the fermentation efficiency under the two methods of pH control.

It can also be seen from Table I that the average ratios of diol to ethanol are somewhat different in the two experiments and that within each experiment there are fluctuations from mash to mash. In the light of considerable experience with this fermentation these differences are not considered significant, and are probably due to such uncontrollable factors as the variability of the organism.

The amount of ammonia required per kilogram of 15% mash has been calculated, and it will be noted that this was appreciably greater in Expt. 1

than in Expt. 2. This is attributed to the relatively ineffective method of pH adjustment in the first experiment and is dealt with further in the section on nitrogen balances.

Table I also shows the total solids in the mashes after fermentation, and, allowing for the carbonate, the values agree quite closely with the theoretical amounts as calculated from the analyses of the wheat.

The following sections deal with the comparative effects of ammonium hydroxide and calcium carbonate on the nature, separation, and recovery of the products and by-products. It should be pointed out that only certain components of the fermented mashes, and the steps involved in their recovery, were considered; others such as the recovery of ethanol and the purification of the diol do not constitute problems affected by the factors under study in this work.

Filtration of the fermented mashes permitted estimation of the relative amounts of filtrate and insoluble solids; the analysis of the fractions provided means of calculating the distributions of the various components. Table II summarizes the data and Table III gives the totals and solids balances for this operation.

TABLE II

SUMMARY OF DATA ON FRACTIONS OBTAINED BY FILTRATION OF FERMENTED MASHES

	Expt. 1			Expt. 2		
	A	B	C	A	B	C
Weight of mash to filter, gm.	6373	6391	6440	5610	5647	5828
Filtrate, total weight, gm.	4257	4367	4310	3660	3707	3793
Diol, %	2.37	2.25	2.43	2.78	2.63	2.73
Solids, %	2.24	2.10	2.43	2.47	2.48	2.97
Kjeldahl nitrogen, %	—	—	—	0.32	0.32	0.20
Ammonia nitrogen, %	—	—	—	0.19	0.19	0.05
Wet solids, total weight, gm.	1700	1665	1780	1720	1770	1715
Dried solids, %	10.52	10.57	13.38	10.34	9.76	13.85
Analysis of dried solids:						
Kjeldahl nitrogen, %	5.55	5.41	4.33	6.10	5.90	4.41
Protein, %, ($N \times 5.7$)	31.6	30.9	24.7	34.8	33.6	25.1
Ash, %	4.53	4.36	18.32	3.46	3.32	25.16

The data in Tables II and III show that the total solids in the fermented mashes were distributed, after filtration, approximately one-third in the filtrates and two-thirds in the filter cakes. This distribution, however, was not truly representative since the filter cakes were not washed. A correction was applied by subtracting from the filter cake solids the weight of the soluble solids in the entrained filtrate. This reduced the average fraction of the total solids in the residues to about 51% and represented true insolubles.

Although the over-all losses in the filtration step may be explained by evaporation, the losses of solids can be attributed only to experimental error.

TABLE III

MATERIAL BALANCES AND DISTRIBUTION OF COMPONENTS IN FILTRATION STEP

	Expt. 1			Expt. 2		
	A	B	C	A	B	C
<i>Totals</i>						
Fermented mash, gm.	6373	6391	6440	5610	5647	5828
Filtrate, gm.	4257	4367	4310	3660	3707	3793
Wet solids, gm.	1700	1665	1780	1720	1770	1715
Material lost, gm.	416	359	350	230	170	320
Loss, %	6.54	5.62	5.44	4.10	2.81	5.00
<i>Solids</i>						
Solids in fermented mash, gm.	284.1	281.2	362.6	280.5	278.0	376.0
Solids in filtrate, gm.	95.4	91.6	104.7	90.2	92.0	112.6
Solids in residue, gm.	178.6	176.0	238.0	177.8	172.5	237.4
Solids lost, gm.	10.1	13.6	19.9	12.5	13.5	25.8
Loss, %	3.56	4.84	5.49	4.46	4.86	6.86
Fraction of solids in filtrate, %	33.7	32.6	28.9	32.2	33.1	29.9
Fraction of solids in residue, %	62.9	62.6	65.6	63.4	62.1	63.2
Insoluble solids in residue, gm.	143.8	144.0	199.5	139.0	131.8	192.2
Fraction of solids as insolubles, %	50.6	51.2	55.0	49.6	47.4	51.2

It is worth noting that the simple filtration method gave a very wet cake. Although 95% of the recovered filtrate was obtained in two to three hours the cake still contained approximately 90% moisture after 12 hr. filtration.

A comparison of the ammonia and carbonate mashes with respect to the distribution of solids in the filtrates and residues shows no significant differences since the slightly larger amounts of insolubles in the carbonate mashes are due to the presence of excess carbonate. Hence it appears that the use of ammonia does not bring about appreciable solubilization of wheat protein. It is also apparent that the total solids in the carbonate mashes are appreciably greater owing to the added carbonate, and, as Table III shows, this increase is reflected in the filtrate, as well as in the residues. This is evidence that some of the carbonate is converted to soluble compounds during fermentation.

Table II shows that the ash is several times higher in the carbonate than in the ammonia residues. This is not unexpected as it is known that 1.0% of carbonate is considerably in excess of the amount required for neutralization of the acid from a 15% mash. The lower protein percentages in the carbonate residues are, of course, a result of the higher ash contents.

The distribution of nitrogen in Expts. 1 and 2, before fermentation and after filtration, is given in Table IV. The data for Expt. 1 are incomplete, but support those of Expt. 2 and hence have been included. This table reveals several interesting facts, of which probably the most significant is the large loss of nitrogen in Expt. 2. This loss is seen to be relatively greater in

TABLE IV
NITROGEN DISTRIBUTION IN FILTRATES AND RESIDUES

	Expt. 1			Expt. 2		
	A	B	C	A	B	C
*Kjeldahl nitrogen from wheat in original mash, gm.	21.0	21.0	20.5	18.2	18.4	19.7
Kjeldahl nitrogen from wheat in inoculum, gm.	0.3	0.3	0.3	0.2	0.2	0.2
Nitrogen added as ammonia, gm.	12.5	12.5	0.0	7.8	8.1	0.0
Total nitrogen supplied, gm.	33.8	33.8	20.8	26.2	26.7	19.9
Filtrate:						
Kjeldahl nitrogen, gm.	—	—	—	11.7	11.9	7.6
Ammonia nitrogen, gm.	—	—	—	7.0	7.0	1.9
Residue:						
Kjeldahl nitrogen, gm.	9.9	9.5	10.3	10.8	10.2	10.5
Loss of Kjeldahl nitrogen, gm.	—	—	—	3.7	4.6	1.8
Loss of Kjeldahl nitrogen, %	—	—	—	14.1	17.2	9.0
Fraction of total nitrogen in residue, %	29.3	28.1	49.5	41.2	38.2	52.8
Fraction of wheat nitrogen in residue, %	46.3	44.6	49.5	58.7	54.9	52.8

* All values corrected for sampling.

the ammonia mashes, *A* and *B*, but is nevertheless appreciable even in the carbonate mash *C*.

Since such losses were not anticipated, nitrogen analyses were not made on the fermented mashes and hence it is not known whether the nitrogen disappeared during fermentation or during the subsequent filtration. It appears most likely, however, that volatile nitrogen compounds were removed during fermentation by the evolved gases, and that such compounds were chiefly ammonia since the losses are substantially higher in the ammonia mashes. The fact that there was a loss in the carbonate mash and that the mash also contained ammonia lends support to this assumption. It is true, of course, that the filtration losses enter into the nitrogen balance, especially as they affect the solids in the residue, but a comparison with Table III shows that the losses there will not account for the nitrogen losses found.

Referring back to the ammonia added to the mashes during fermentation (Table I), it now appears reasonable to attribute the larger amounts of ammonia needed in Expt. 1 to a relatively greater loss. This can be explained by the relatively ineffective method of pH control, which caused the pH to fluctuate considerably above the desired level of 6.5. If ammonia were carried out by the fermentation gases then the losses would obviously be greater at higher pH levels. These observations lead to the conclusion that the theoret-

ical amount of ammonia required per kilogram of 15% mash is considerably less than 1.65 gm. The practical significance of this is discussed in a later section of this paper.

Another point illustrated by the data of Table IV is that only about half of the original wheat nitrogen appears in the solids of the residue. The rest was solubilized and, as the carbonate mash shows, partly converted to ammonia.

It should be pointed out that the omission of washing of the filter cakes affected the distribution of nitrogen between filtrates and residues. A correction for the nitrogen in the entrained filtrate can be applied similarly to that mentioned above for the distribution of solids. This will decrease the nitrogen fractions in the residues by approximately 20% of the values shown in Table IV.

Simple distillation of the filtrates at atmospheric pressure enabled an estimate to be made of the quantity of syrup remaining after removal of most of the aqueous phase. This syrup results from accumulation of the soluble solids in the filtrate. Since it is susceptible to thermal decomposition when its solid content approaches 50% it is impossible to remove the remaining liquid which contains a high percentage of the total diol. Since the handling of this syrup is a difficult technical problem it is desirable to reduce the volume to a minimum.

The results of the distillation step are shown in Table V. It is apparent from the results that there was no appreciable difference in the amounts of syrup formed in the filtrates from the ammonia and carbonate treated mashes, nor were any physical differences observed in the various syrups. Furthermore, this lack of significant variation under the two treatments is reflected in the distribution of diol between the distillates and syrups. The differences shown in the data may be attributed to lack of uniform conditions, e.g., a slight amount of reflux occurring in the distilling head, and variable initiation of decomposition owing to point heating of the flask.

TABLE V
DISTRIBUTION OF COMPONENTS BY DISTILLATION OF FILTRATES

	Expt. 1			Expt. 2		
	A	B	C	A	B	C
Weight of filtrate distilled, gm.	2400	1900	2127	2107	1921	1930
Weight of distillate, gm.	2265	1815	2035	1970	1811	1790
Weight of syrup, gm.	148	82	91	127	101	118
Weight of material lost, gm.	-13	3	1	10	9	22
Weight of syrup						
Weight of filtrate, %	6.2	4.3	4.3	6.0	5.2	6.1
Solids in syrup, %	33.3	47.1	51.0	41.2	48.1	45.2
Fraction of original diol in syrup, %	55.2	60.0	55.3	64.5	72.5	68.5

Fermentations at Different pH Levels

It has been shown in Expts. 1 and 2 that the pH of whole wheat mash fermented with *A. polymyxa* may be satisfactorily controlled by addition of ammonia. Since the use of ammonia permitted adjustment of the pH to any desired level it became of interest to compare fermentations carried out at various pH's within the range 5.6 to 7.0.

Six-litre quantities of 15% mash were prepared, inoculated, and fermented as previously described. The wheat used in this experiment had the following analysis: moisture, 13.2; starch, 55.1; nitrogen, 2.3; protein, 13.1% (N \times 5.7). Duplicate mashs were fermented at each of three pH levels: 6.0, 6.5, and 7.0; pH adjustment by the electrometric method was made at eight-hour intervals. Duplicate mashs containing 1.0% of calcium carbonate served as controls, and at the end of each 24 hr. period samples were taken from all mashs for chemical analyses.

Table VI shows the amounts of diol and ethanol formed at various times in the fermenting mashs. For purposes of comparison all quantities have been calculated on the basis of a 15% mash, and the tabulated results are the means of the duplicates.

TABLE VI
COMPARATIVE EFFECTS ON PRODUCTS YIELD OF CONTROLLING pH WITH
AMMONIA AND WITH CALCIUM CARBONATE

Time, hr.	--	Ammonia			1% Calcium carbonate
		pH = 6.0	pH = 6.5	pH = 7.0	
24	Diol, %	0.63	0.46	0.61	0.83
	Ethanol, %	0.40	0.31	0.43	0.54
48	Diol, %	1.93	1.71	1.83	1.94
	Ethanol, %	1.15	1.12	1.12	1.19
72	Diol, %	2.54	2.57	2.55	2.61
	Ethanol, %	1.66	1.67	1.65	1.66
96	Diol, %	2.73	2.75	2.62	2.75
	Ethanol, %	1.75	1.73	1.68	1.75
120	Diol, %	2.75	2.79	2.66	2.80
	Ethanol, %	1.81	1.77	1.80	1.84

It will be seen from the data of Table VI that the fermentation rates of mashs controlled at the various pH levels by ammonia do not differ appreciably either among themselves or from the rate in the carbonate controlled mash. A possible exception may be the decreased rate of diol formation at pH 7.0 after 72 hr.

The fermentation efficiencies and diol-ethanol ratios, for the various pH levels, are tabulated in Table VII. The ratio was remarkably constant not only at the different pH levels but also at the various fermentation times,

TABLE VII

THE COMPARATIVE EFFECTS ON DIOL-ETHANOL RATIO AND ON FERMENTATION EFFICIENCY OF CONTROLLING pH WITH AMMONIA AND WITH CALCIUM CARBONATE

pH	Time in hours							
	Diol-ethanol ratio					Fermentation efficiency, %		
	24	48	72	96	120	72	96	120
5.8 (carbonate)	1.55	1.62	1.58	1.57	1.52	93.8	99.4	102.2
6.0	1.57	1.67	1.53	1.55	1.52	92.8	98.6	100.4
6.5	1.52	1.52	1.53	1.59	1.57	93.7	98.8	100.4
7.0	1.43	1.64	1.54	1.56	1.48	92.5	94.9	98.4

although the lower ratio at pH 7.0 and 120 hr. may be significant. The relatively greater variation in ratios at the 24 and 48 hr. periods seems to be an inherent characteristic of this fermentation. However, the actual level of the ratio in this experiment is of little significance since it has been shown that it is governed largely by the degree of aerobiosis (1).

The fermentation efficiencies were calculated from 72 hr. on since only at this time were the fermentations sufficiently complete to be of practical interest. These results show that at the various levels the fermentations approached completeness at approximately the same rate. As previously mentioned, the fermentation at pH 7.0 may be an exception. In general, it may be concluded from these data along with those of Expts. 1 and 2 that variation in the pH in the range 5.6 to 7.0 has little effect on the fermentation efficiency.

As previously pointed out, the method of pH control did not achieve as precise regulation as was desired. In Fig. 1 the mean pH values for each eight-hour period are plotted, and for comparison the course of the pH in the mash containing calcium carbonate is included. The relatively large fluctuations in the early stages of the fermentations indicated the formation of surprisingly large amounts of acid. More effective control would have necessitated more frequent sampling; this would have increased the dangers of contamination and was undesirable from the standpoint of depleting the mash volumes. It is felt, however, that with the possible exception of the mashes at pH 7.0 sufficient control was established to permit valid assessment of the effect of the different pH levels. As previously shown in Table VII, the mashes that were intended to be at pH 7.0 showed a decreased rate of fermentation after 72 hr. Since Fig. 1 shows that only during this period did the pH approximate the desired level it is reasonable to suppose that had a pH of 7.0 been maintained throughout, the over-all rate of fermentation would have been correspondingly decreased; in addition, larger amounts of acid might have been expected.

In Fig. 2 are plotted the mean volumes of 1.48 *N* ammonium hydroxide solution required to adjust 50-ml. samples of the mashes to the prescribed pH levels at the end of each eight-hour period. These quantities are an indication of the acid formed in each period. Two significant facts are shown by these

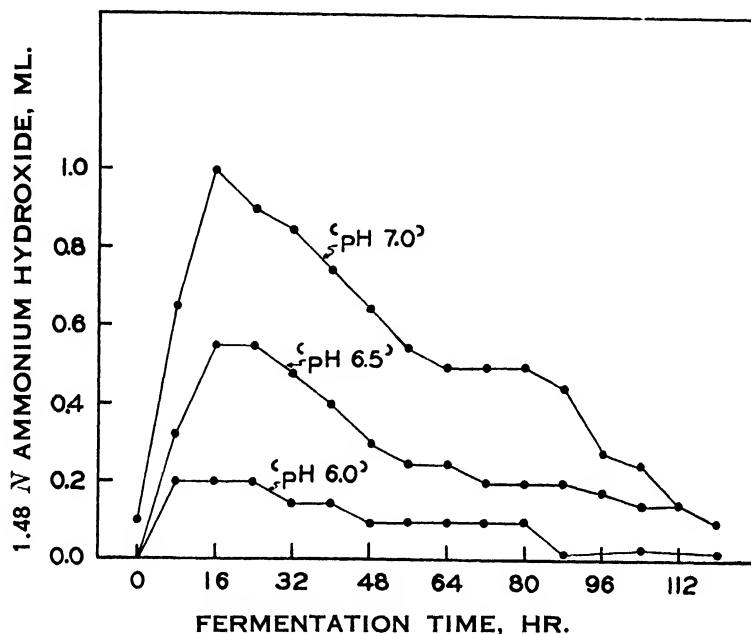


FIG.2. Ammonium hydroxide required to adjust 50-ml. aliquots of mash to prescribed pH levels during fermentation.

curves. First, the production of acid was greatest during the first 24 hr. of fermentation, thus confirming the conclusion made from Fig. 1; and secondly the higher the pH level the greater was the amount of acid produced. The latter observation was proved by measuring the total acids in the fermented mashes at 120 hr. The results, calculated as acetic acid, are given in Table VIII.

TABLE VIII

THE COMPARATIVE EFFECTS ON ORGANIC ACID YIELD OF CONTROLLING pH WITH AMMONIA AND WITH CALCIUM CARBONATE

Neutralizing agent	pH	Acetic acid, %	Acetic acid, moles per litre of fermented mash
Carbonate	5.8	0.242	0.0403
Ammonia	6.0	0.294	0.0490
Ammonia	6.5	0.385	0.0642
Ammonia	7.0	0.667	0.1112

Materials Balance

Table IX gives the materials balance for the fermentations of Expt. 3. The weights of dry gases given off during the fermentations were calculated by subtracting the true weights of the fermented mashes from the total weights of the original mashes plus inoculum plus the concentrated ammonium hydroxide added. The true weight of each fermented mash was arrived at

TABLE IX
SUMMARY OF DATA ON FERMENTATIONS AT DIFFERENT PH LEVELS
(All data are the means of duplicates)

Neutralizing agents	Carbonate	Ammonia		
pH of mash	5.8	6.0	6.5	7.0
Initial weight of mash, gm.	5770*	5827	5790	5050
Wheat in mash, %	15.31	15.41	15.56	15.63
Weight of inoculum, gm.	203	203	203	203
Actual weight of fermented mash, gm.	4330	4348	4350	3641
**True weight of fermented mash, gm.	5743	5765	5775	5124
Solids in actual fermented mash, %	6.25	4.86	4.39	4.70
†Weight of ammonium hydroxide added, gm.	0.00	16.64	45.52	75.09
†Weight of ammonia added per kgm. of 15% original mash, gm.	0.00	0.78	2.12	3.99
†Moles of ammonia added per litre of fermented mash	0.000	0.0476	0.1302	0.2414
Weight of dry gas evolved, gm.	290	282	267	204
Weight of dry gas per kgm. of 15% mash, gm.	49.2	47.1	44.5	38.7
Kjeldahl nitrogen in actual fermented mash, %	0.367	0.406	0.475	0.568
Ammoniacal nitrogen in actual fermented mash, %	0.011	0.030	0.098	0.166

* Sixty gm. of calcium carbonate not included.

** See text for meaning of true weight.

† Corrected for mash sampling.

by correcting for the following: samples removed, gases and ammonium hydroxide associated with samples, water vapour removed by the fermentation gases, and finally the ammonia lost during the fermentations.

It will be noted from Table IX that the weight of evolved gases decreases with increasing pH, and this suggests a correlation with the increase in total acids shown in Table VIII. The nature of this relation is made clear by the carbon balance in Table X. The close agreement between the total amounts of carbon before and after fermentation indicates that all the main products were satisfactorily accounted for.

It will be noted that, excepting the carbonate mash, the apparent loss in carbon increases with the pH. This is due probably to two causes. The acids were calculated as acetic, but it is known from previous experiments that a portion of the total acid is lactic. Hence the average carbon per mole

TABLE X

CARBON BALANCES FOR FERMENTATIONS AT DIFFERENT pH LEVELS

Basis: 37.2 gm. of carbon from 1 kgm. of 15% wheat mash*

Neutralizing agent	Carbonate	Ammonia		
pH of mash	5.8	6.0	6.5	7.0
Weight of carbon in:				
**Diol, gm.	14.41	14.05	14.38	13.90
Ethanol, gm.	9.28	9.05	8.94	9.26
† Totals acids, gm.	0.94	1.11	1.45	2.55
Carbon dioxide, gm.	13.16	12.60	11.91	10.33
Total carbon accounted for, gm.	37.79	36.81	36.68	36.04
Loss in carbon, gm.	-0.59	0.39	0.52	1.16
Loss in carbon, %	-1.61	1.05	1.40	3.12

* Includes carbon from inoculum starch. Assumed that starch was only source of fermentables and was totally fermented.

** Includes acetoin.

† Calculated as acetic acid.

of acid would be greater than that calculated and the error from this factor would be greater with greater amounts of acid. Again, the dissolved carbon dioxide remaining in the mashes at the end of fermentation was not determined. This, too, would be greater at higher pH levels. The slight excess of carbon found in the carbonate mash may be attributed to a partial release of the carbon dioxide from the calcium carbonate.

Since the diol plus ethanol yields are relatively constant at the different pH levels it must be concluded that the increase in acid at the higher pH levels occurs at the expense of decreased carbon dioxide production. This is noteworthy since it is known that in a normal *A. polymyxa* fermentation (controlled with calcium carbonate) almost 50% of the glucose is converted to carbon dioxide. Hence, it appears that without appreciably altering the yield of diol plus ethanol or the over-all rate of fermentation the chemical balance of the system may be shifted by variation of pH.

From the data in Table IX it was possible to make nitrogen balances on the four fermentations. These are summarized in Table XI, and show a progressive loss in nitrogen (as ammonia) with increasing pH. Losses of this order were previously found in Expt. 2, but at that time they could not be definitely attributed to the fermentation step. The present evidence shows that the theoretical amounts of ammonia required per kilogram of 15% mash are less than those actually found. In fact, the data in Tables IX and XI show that approximately twice as much ammonia was added as remained fixed in the mashes after fermentation. It would be expected that the amounts of ammonia required to maintain the pH of the mash at the prescribed levels would be almost chemically equivalent to the acids formed.

TABLE XI

NITROGEN BALANCE FOR FERMENTATIONS AT DIFFERENT pH LEVELS*

Neutralizing agent	Carbonate	Ammonia		
pH of mash	5.8	6.0	6.5	7.0
Kjeldahl nitrogen in original mash, gm.	20.35	20.65	20.71	18.17
Kjeldahl nitrogen in inoculum, gm.	0.23	0.23	0.23	0.23
Ammonia nitrogen added, gm.	0.00	3.84	10.50	17.32
Total nitrogen supplied, gm.	20.58	24.72	31.44	35.72
Kjeldahl nitrogen in fermented mash, gm.	21.05	23.40	27.40	29.10
Ammonia nitrogen in fermented mash, gm.	0.63	1.73	5.65	8.52
Non-ammonia nitrogen in fermented mash, gm.	20.42	21.67	21.75	20.58
Ammonia in fermented mash, moles/litre	0.0080	0.0218	0.0711	0.1203
Loss in ammonia nitrogen, gm.	-0.63	2.11	4.85	8.80
Loss in non-ammonia nitrogen, gm.	0.16	-0.79	-0.81	-2.18

* All values corrected for sampling.

A comparison of the data in Tables VIII and XI reveals that this assumption is justified.

As previously observed in Expt. 2 a small amount of ammonia was found in the carbonate controlled mash. This undoubtedly arose from proteolytic breakdown of the gluten fraction. The apparent increases in the non-ammoniacal nitrogen contents of the mashes controlled with ammonia suggest that some of the added ammonia was converted to other nitrogen compounds; further investigation is required to prove this point.

Vitamin Contents of Fermented Mashes

Although it was not considered likely that the pH of the fermenting mashes would affect their vitamin contents it seemed desirable to supplement the material balances already made with estimates of those vitamins most likely to be unaffected by the recovery process. Such vitamins are important if the unfermented solids are to be recovered as feed.

Attention was restricted to riboflavin and nicotinic acid, both of which are relatively thermostable. Since these vitamins are water soluble, determinations were made on the unfiltered mashes of Expt. 3.

The ultimate recovery of both the soluble and insoluble feed fractions involves heat treatments and it is important therefore to know the stability of the vitamins under such conditions. This was tested by slow evaporation of the whole mashes at atmospheric pressure, under reflux. The evaporations took about 10 hours and reduced the volumes by approximately 75%; the temperature of each mash ranged from 99° to 105° C. The residues were then made up to their original volumes and reanalysed for riboflavin and

nicotinic acid. The results are given in Table XII and include, for comparison, the corresponding contents of the original wheat.

TABLE XII

RIBOFLAVIN AND NICOTINIC ACID CONTENTS OF MASHES BEFORE AND AFTER
FERMENTATION AND AFTER HEAT TREATMENT

Basis: 100 gm. of fermented mash

Neutralizing agent	pH of mash	Original wheat		Fermented mash		Heat treated mash	
		Ribo-flavin	Nicotinic acid	Ribo-flavin	Nicotinic acid	Ribo-flavin	Nicotinic acid
Carbonate	5.8	19.0	1250	30.4	706	28.6	710
Ammonia	6.0	19.4	1280	39.8	815	39.0	780
Ammonia	6.5	19.4	1280	32.3	785	30.1	764
Ammonia	7.0	19.2	1265	34.3	755	33.7	776
Means	—	19.25	1269	34.2	765	32.9	758

All values expressed as μ gm.

The data show that the heat treatment caused no significant losses of either vitamin. In the fermentation process itself, however, an 80% increase in riboflavin and a 40% decrease in nicotinic acid are indicated. Since the total solids remaining after fermentation (on a dry basis) are roughly one-third of the original wheat (on an "as is" basis) it follows that the concentrations of these vitamins in the dried solids would be approximately 5.4 and 1.8 times, respectively, their concentrations in the wheat. The amounts of the above vitamins in the yeast extract of the inoculum were not great enough to affect significantly the results. Little significance is attached to the variations between mashes at different pH levels.

Application to Large Scale Process

The laboratory investigations described in this paper provide several results, which, it is felt, will prove applicable to the large scale process.

It has been shown feasible to carry out the fermentation using ammonia as a means of controlling pH, and for several reasons the range of pH 5.8 to 6.0 is most favourable. At this lower level the amount of ammonia required, the escape of ammonia from the mash, and the production of acid are all minimized. It might be pointed out that although the increased acid production at higher pH levels means a more efficient utilization of the starch, such an advantage would be more than offset by the difficulties of recovering the products.

It is obvious from Fig. 1 that automatic injection of the ammonia will be necessary to maintain the pH closer to the prescribed level, and hence the amounts of ammonia required per unit of mash will be somewhat greater

than those calculated in Table IX. On the other hand, the losses of ammonia could probably be reduced by introducing it below the level of the mash.

If a figure of 1.0 gm. per kgm. of 15% mash is taken as the ammonia requirement at pH 6.0 a simple calculation shows that the amount per bushel of wheat (60 lb.) fermented will be a 0.40 lb. The corresponding amount of calcium carbonate (as 1.0% in the mash) is 4.0 lb. At 20c. per lb. for anhydrous ammonia and 4c. per lb. for calcium carbonate the costs per bushel of wheat would be 8 and 16c., respectively. It has been shown recently that the fermentation will proceed satisfactorily with 0.5% of carbonate, and on this basis the costs of the two chemicals become equal. In addition to the above the use of ammonia will avoid the handling and mixing of a powdered solid.

A further advantage in the use of ammonia is the production of solid residues of very low ash content as compared with the residues from carbonate mashes. Since the nitrogen content is unimpaired these residues should have greater values as feed.

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PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

IX. THE EFFECT OF VARIOUS NUTRIENT MATERIALS ON THE FERMENTATION OF STARCH BY *AEROBACILLUS POLYMYXA*¹

BY SYBIL B. FRATKIN² AND G. A. ADAMS²

Abstract

Wheat starch is a poor medium for fermentation by *Aerobacillus polymyxa*. The solubles recovered from the separation of starch and gluten in patent flour enhance the fermentation but not as effectively as the similar fraction from whole wheat flour. Addition of supplements is necessary for a satisfactory yield of products in a reasonable length of time. Wheat gluten has no stimulatory effect but bran and shorts are both effective, the latter being slightly superior. An 8% starch medium fortified with the solubles from whole wheat required a 2.5% supplement of shorts to bring fermentation by *A. polymyxa* to 90% completeness in 72 hr.

Of the various supplements tested, a 1% addition of malt sprouts proved to be the most effective, fermentation being 90% complete in 72 hr. Shorts, bran, Cerogras (dehydrated young oats), alfalfa, soya beans, yeast extract, and corn-steep liquor follow in order of decreasing effectiveness.

The solubles from whole wheat when ashed have no beneficial effects on the fermentation of starch by *A. polymyxa*.

Introduction

During the course of investigations on the production of 2,3-butanediol by fermentation of whole wheat with *Aerobacillus polymyxa*, it was found that the recovery of diol by distillation was hindered as evaporation progressed by accumulation of soluble solids in the form of a syrup. It has been shown that a large proportion of the soluble solids originate from solubilized wheat protein (1). It seemed advantageous, therefore, to remove the bulk of the wheat protein (gluten) prior to the fermentation provided its absence did not seriously interfere with the efficiency of the fermentation. Preliminary evidence already existed that most of the gluten could be removed without adversely affecting the fermentation (6). A satisfactory process for the separation of starch and gluten from patent flour had been developed in these laboratories (8). When used with whole wheat flour, this process removed the bran and shorts with the gluten and left a starch slurry containing soluble fractions of the wheat. From preliminary experiments it was apparent that the starch + the water soluble fraction of wheat ("wash water") was not a satisfactory fermentation medium for *A. polymyxa* and that it was necessary to fortify this substrate with additional supplements.

Recent researches in bacterial nutrition have established the importance of certain accessory substances for growth as well as fermentation. Growth factors for *A. polymyxa* have not been worked out completely as yet, but

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biotin has been shown to be essential, whereas thiamin, which stimulated growth of some strains, inhibited others. Pantothenic acid, nicotinic acid, riboflavin, pyridoxine, and inositol were ineffective as nutrilites (5). Yeast extract as a nutrient source for *A. polymyxa* has been reported previously (4, 6) but for large scale operations use of either pure nutrient materials or yeast extract is not practicable and a cheaper source of nutrients is desirable.

A number of cheaper supplements have been used with success in other fermentations. Tatum, Peterson, and Fred (11) used cabbage, oranges, yams, potatoes, alfalfa, soya beans, wheat middlings, and malt sprouts to increase the production of butyl alcohol in the fermentation of corn-mash by certain butyric acid bacteria. Corn-steep liquor was used to supply necessary nutrients for *Acetobacter suboxydans* (10) and *Lactobacillus delbrückii* (9). Recently Fulmer, Bantz, and Underkofler (3) reported use of alfalfa extract as a nutrient supplement for growth and chemical activity of *A. suboxydans*.

The purpose of the present investigation was to find suitable supplements for a starch fermentation by *Aerobacillus polymyxa*.

Materials and Methods

Three types of starch mashes were used; starch-whole-wheat-wash-water, starch-patent-flour-wash-water, and commercial-wheat-starch-tap-water. The process for separating starch and gluten developed in these laboratories (8) was applied to whole wheat and patent flours. The final concentration of starch was adjusted to 8.0 to 8.25% so that the yields could be compared with those from 15% whole wheat mashes. The starch suspensions were preliquefied for 20 min. at 70° C. with 1% barley malt (on the basis of the weight of wheat that would contain the starch present), and cooked at 100° C. for five minutes. Three hundred millilitres of mash was dispensed into 500 ml. Erlenmeyer flasks containing calcium carbonate (3 gm.) and the nutrient supplement being tested. After sterilization for one hour at 15 lb. pressure, followed by cooling to 30° C., 10 ml. of a 24 hr. culture was added. For all experiments reported here a locally isolated strain of *A. polymyxa* designated as N.R.C. 3 (2) was used. All fermentations were carried out at 30° C.

In most experiments, fermentations were allowed to proceed for 48, 72, 96, and 120 hr. A separate flask was set up for each time interval so as to avoid introducing contaminants during sampling.

Methods of analysis for butanediol and alcohol have been described in a previous paper (6).

Experimental Results

Effect of Gluten on Fermentation of Wheat by Aerobacillus polymyxa

Gluten was washed out by hand (2) from a sample of whole wheat flour, drum dried, and milled to 60 to 80 mesh. The gluten was added back in varying percentages of the original amount present to a mash made of the residual starch, bran, and wash water. Results of fermentation are given in Fig. 1. Only curves for total products are shown since the diol-ethanol ratio

was not affected by different amounts of gluten. It is apparent that gluten had very little effect on the yield of product. A small amount (10 to 25%) did increase the yield slightly in the early stages but this effect disappeared as fermentation neared completion. Controls of whole wheat mash always

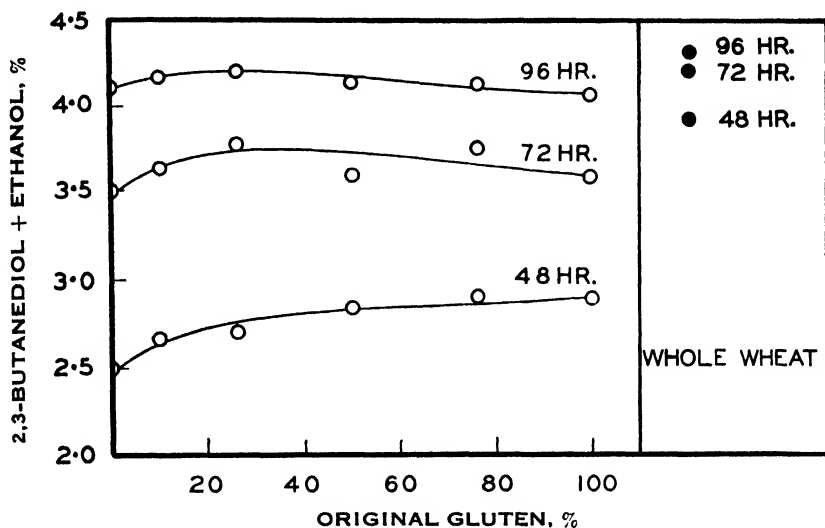


FIG. 1. Effect on fermentation of adding gluten to a medium of starch, bran, and wash water in comparison with a whole wheat medium of the same starch content.

gave slightly higher yield of product than even the best of the reconstituted mashes, but this is explicable on the basis of a slightly lower starch content of the latter. In addition, the rate of fermentation in whole wheat mashes was much greater than in the reconstituted mashes containing all the original gluten.

Effect of Bran, Shorts, and Wheat Germ in Fermentation of Starch

Since whole wheat mashes were fermented satisfactorily, it seemed desirable to assess the values of the bran, shorts, and wheat germ fractions as nutrient supplements in a starch fermentation. The effect of each fraction was determined on the three types of starch mashes previously described.

While the bran and shorts fraction of wheat is subject to considerable variation in quantity, it may be regarded as 27% of the wheat. The relative amount of each depends largely on the milling process but is usually equal. Thus in 300 ml. of a 15% whole wheat mash (8.25% of starch), there would be 12.15 gm. of bran + shorts, i.e., approximately 4% of the weight of the mash. Wheat germ content of whole wheat is 1 to 2% and in a 15% wheat mash there would be approximately 0.3 gm. per 100 ml. of mash.

Preliminary experiments showed that bran, shorts, and wheat germ were very similar in their stimulatory action on the fermentation of starch by *A. polymyxa*. However, addition of all the wheat germ originally present in the wheat was no more effective than the same amount of bran or shorts.

TABLE I
EFFECT OF WHEAT GERM, BRAN, AND SHORTS AS NUTRIENT SUPPLEMENTS
ON FERMENTATION OF STARCH

Nutrient	%	2,3-Butanediol + ethanol, %			
		48 hr.	72 hr.	96 hr.	120 hr.
Control		1.16	1.52	1.84	2.11
Wheat germ	0.1	1.23	1.73	1.94	2.41
Bran	0.1	1.30	1.66	2.06	2.48
Shorts	0.1	1.18	1.68	2.00	2.53
Control		1.16	1.52	1.84	2.11
Wheat germ	0.3	1.31	1.66	2.31	2.72
Bran	0.5	1.64	2.22	2.45	3.06
Shorts	0.5	1.58	2.14	2.66	2.76

Hence, use of wheat germ for commercial production would not be feasible because of its higher cost. A typical result, showing the interchangeability of bran, shorts, and wheat germ as supplements is given in Table I.

Since it would be more practical to add either all bran or all shorts in a large scale operation, amounts up to and including the total amount present in a 15% whole wheat mash of both bran and shorts were used. These amounts were equivalent to 4% of the weight of the mash. An experiment was done in which both bran and shorts were added in varying proportions ranging from all bran to all shorts. The results showed that shorts was slightly superior to bran and that there was nothing specific in the action of either, but rather that their stimulatory effects were additive (Table II).

TABLE II
EFFECT OF BRAN + SHORTS IN VARYING PROPORTIONS ON FERMENTATION OF STARCH

Supplement*	2,3-Butanediol + ethanol, %			
	48 hr.	72 hr.	96 hr.	120 hr.
100% Bran	2.50	4.01	4.14	4.24
75% Bran + 25% shorts	2.60	3.99	4.21	4.29
50% Bran + 50% shorts	2.51	4.07	4.23	4.20
25% Bran + 75% shorts	2.72	4.16	4.18	4.19
100% Shorts	2.93	4.10	4.21	4.28

*A supplement of 4% of the weight of the mash was used in each case.

(1) *Mash, Starch-Whole-wheat-wash-water*—Effects of bran and shorts in a mash fermentation on starch mixed with the wash water from whole wheat are shown in Fig. 2. Comparison of the two sets of curves shows that shorts gave slightly higher total products than bran but this effect can be explained partially on the basis of higher starch content of shorts (shorts 11.32%, bran

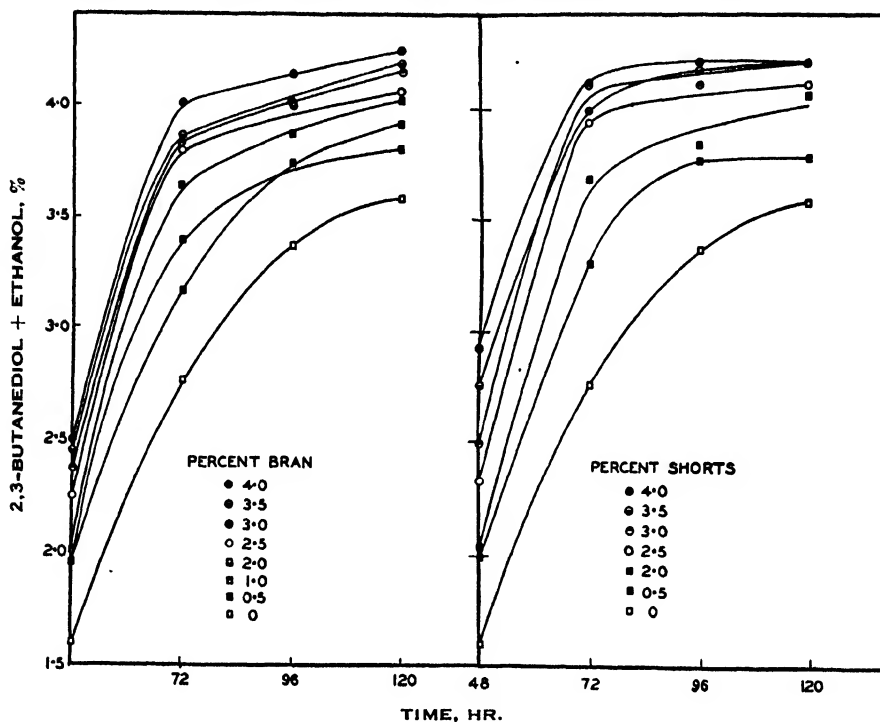


FIG. 2. Effect of various amounts of bran and shorts on fermentation of a starch-whole-wheat-wash-water mash.

7.88%). With either supplement, the rate of fermentation increased with increase in nutrient added. This effect was more noticeable at the lower concentrations where a slight increase in supplement caused a marked increase in rate. However, a saturation point for supplement is reached where further amounts gave only slightly higher yields, which were due principally to the additional starch supplied by the supplement. It follows, therefore, that for this mash, 2.5% of either bran or shorts was the optimum concentration.

Using 2.5% of bran and shorts, fermentations were 88 and 90% complete, respectively, at 72 hr. These percentages were calculated on the basis of a 7.64% starch content of the mash and corrections were applied for the starch in the nutrient supplement. It is interesting to note that starch and wash water alone fermented fairly well but the fermentation did not reach completion even after 144 hr. This indicates that the wash water either did not contain some essential factor or was lacking in sufficient quantities of the necessary nutrients to carry fermentation to completion.

(2) *Mash, Starch-Patent-flour-wash-water*—Fig. 3 shows the effects of bran and shorts in a starch-patent-flour-wash-water mash. In this experiment also, shorts gave an over-all higher yield of products than bran. A comparison with Fig. 2 shows that the rate of fermentation was much slower for this mash than in the whole-wheat-wash-water-starch mash since the curves

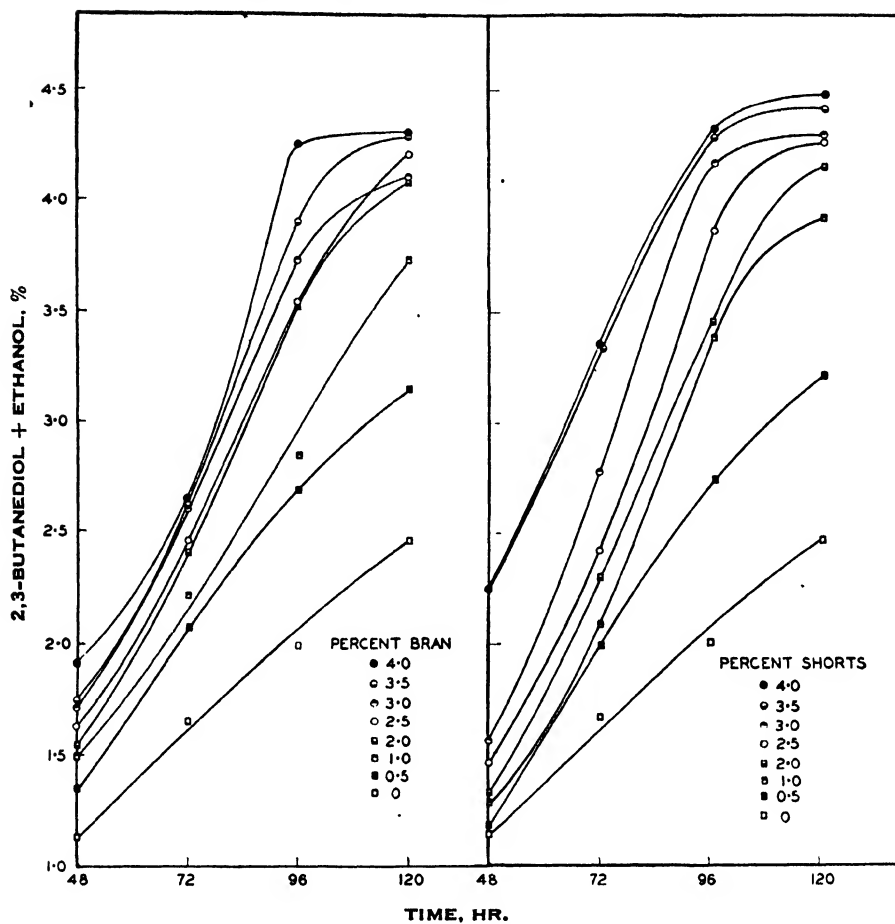


FIG. 3. Effect of various amounts of bran and shorts on fermentation of a starch-patent-flour-wash-water mash.

in the former do not begin to flatten out till 96 hr. In addition, at least a 3% supplement was required to bring the fermentation to completion. With a 3% supplement of bran and shorts, fermentations at 96 hr. were 81 and 89% complete, respectively. The starch and wash water alone was fermented rather poorly and at 120 hr. fermentation was only 55% complete.

(3) *Mash, Commercial-starch-Tap-water*—Effects of bran and shorts on the fermentation of a mash of commercial starch and tap water are shown in Fig. 4. Again shorts increased the rate of fermentation slightly more than bran. The control mashes of starch and tap water were fermented very poorly and at 120 hr. fermentations were only 25% complete. Addition of small amounts of supplement was markedly beneficial but a 3 to 3.5% supplement of either bran or shorts was necessary for optimum results. With 3% of either supplement fermentations were 88% complete at 96 hr.

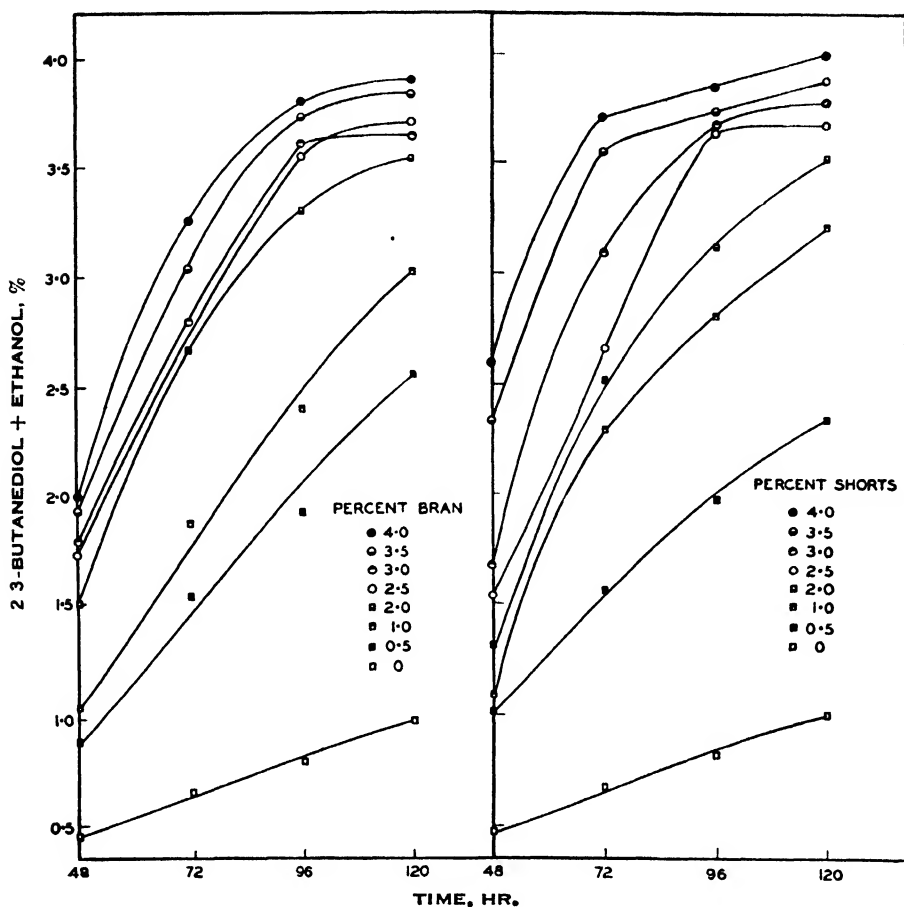


FIG. 4. Effect of various amounts of bran and shorts on fermentation of a starch-lap-water mash.

Effect of Other Supplements

In addition to bran, shorts, and wheat germ a number of other substances in varying concentrations were used as nutrient supplements in a starch-whole-wheat-wash-water mash. These included yeast extract, corn-steep liquor, malt sprouts, unsterilized malt sprouts, alfalfa, soya beans, and Cerogras*. For comparison, bran and shorts were also included in the series. The results given in Table III were corrected for fermentables in the added nutrients.

Of the various supplements tested, malt sprouts gave the best results, fermentation being 90% complete in 72 hr. with only 1% of this supplement. Unsterilized malt sprouts have been successfully used as a supplement for *Lactobacillus delbrückii* in a lactic acid fermentation (7). Unsterilized malt sprouts in the *Aerobacillus polymyxa* fermentation gave even better results than sterilized sprouts, but the danger of introducing contamination more

* Cerogras—dehydrated young oat plants.

TABLE III

EFFECT OF VARIOUS SUPPLEMENTS IN VARYING CONCENTRATIONS IN A STARCH-WHOLE-WHEAT-WASH-WATER MASH* FERMENTATION

Nutrient	%	2,3-Butanediol + ethanol, %		
		48 hr.	72 hr.	96 hr.
Control		1.61	2.99	3.52
Malt sprouts**				
Sterilized	1.0	2.95	4.03	4.21
Unsterilized	0.5	3.43	0.76†	0.80†
Shorts‡	1.0	2.01	3.64	4.11
Bran§	1.0	2.19	3.57	4.16
Cerogras††	1.0	2.64	3.61	4.03
Alfalfa	1.0	2.49	3.87	4.06
Soya beans	1.0	1.48	3.13	3.80
Yeast extract	0.5	2.72	3.75	3.89
Corn-steep liquor	0.5	2.09	3.13	3.54
Malt sprouts				
Sterilized	2.0	3.14	4.25	4.45
Unsterilized	1.0	3.61	4.24	4.11†
Shorts	2.0	1.95	3.80	4.23
Bran	2.0	2.03	3.68	4.22
Cerogras	2.0	2.85	3.85	4.18
Alfalfa	2.0	2.94	3.77	4.01
Soya beans	2.0	2.03	3.58	4.12
Yeast extract	1.0	2.88	3.75	3.96
Corn-steep liquor	1.0	2.47	3.51	3.80
Malt sprouts				
Sterilized	3.0	3.44	4.36	4.48
Unsterilized	1.5	3.37	2.40†	2.39†
Shorts	3.0	2.24	4.11	4.38
Bran	3.0	2.05	3.54	4.22
Cerogras	3.0	2.90	3.87	4.17
Alfalfa	3.0	3.17	4.15	4.21
Soya beans	3.0	1.99	3.64	4.06
Yeast extract	1.5	2.87	3.58	3.95
Corn-steep liquor	1.5	1.93	3.16	3.57

* Starch content of mash, 7.93%.

** Fermentables in malt sprouts, 22.59%.

• † Contaminated.

‡ Starch content of shorts, 11.32%.

§ Starch content of bran, 7.88%.

†† Cerogras—dehydrated young oat plants; obtained from Greenmelk Co., Ltd., Wallaceburg, Ont.

than outweighed the beneficial effects. In several experiments an attempt was made to overcome possible contaminants by addition of large amounts of inoculum (three or five times the amount usually employed), but again increase in yield did not compensate for the additional inoculum required. Next in order of effectiveness were shorts and bran, both of which, in a 3% concentration gave a fermentation approximately 90% complete in 72 hr. When used in their highest concentration, Cerogras, alfalfa, soya beans, yeast extract, and corn steep liquor followed in order of decreasing effectiveness.

Effect of Ash of Wash Water

Wash water obtained from a whole wheat flour by the starch separation process was taken to dryness on a steam-bath and then ashed in a furnace at 550° C. to constant weight. The ash was then taken up in its original volume of tap water and slightly acidified with sulphuric acid. Mashers were prepared with the appropriate amounts of starch and fermented in the usual manner. Control mashers of starch-tap-water and starch-whole-wheat-wash-water were fermented at the same time. Results given in Table IV showed that ashing destroyed the growth promoting factors (4).

TABLE IV

EFFECT OF WHOLE WHEAT WASH WATER AND ITS ASH IN FERMENTATION OF STARCH

Description of mash	2,3-Butanediol + ethanol, %		
	48 hr.	72 hr.	96 hr.
Starch + ash + tap water	0.54	0.69	0.86
Starch + tap water	0.63	0.90	0.98
Starch + whole wheat wash water	1.73	2.32	3.01

Discussion

From the experimental results it is apparent that with suitable nutrient supplements *A. polymyxa* can ferment 8% starch mashers satisfactorily. It was not the purpose of this investigation to fractionate out any specific growth factor or factors but rather to find a readily available supplement containing the necessary factors to bring about a satisfactory fermentation of starch. Whole wheat wash water which itself supported growth to some extent had no growth promoting effect when ashed. This suggests that the growth factors are organic in nature but does not exclude the possible need for certain inorganic salts as well.

A starch-whole-wheat-wash-water mash with a supplement of 1% of malt sprouts or 2.5% of shorts appears to be the best material for commercial production. In normal times, malt sprouts are as cheap as shorts and as readily available. A starch-patent-flour-wash-water mash with a slight increase in supplement and time of fermentation might be also used as a fermentation medium. In this instance the by-product from the separation process would be a relatively pure gluten, which has a much higher market value than the gluten-bran feed from a whole wheat flour separation. The question as to whether whole wheat or patent flour could be most profitably used is largely an economic one.

Acknowledgments

The authors wish to thank Mr. A. L. Shewfelt for his assistance in carrying out the starch separations from whole wheat and patent flours used in the course of this work.

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DRIED WHOLE EGG POWDER

XVIII. THE KEEPING QUALITY OF ACIDULATED, GAS-PACKED POWDERS OF LOW MOISTURE CONTENT¹

BY JESSE A. PEARCE², MARGARET REID², AND W. H. COOK³

Abstract

Acidification of liquid egg prior to drying did not improve subsequent storage life, although pH measurements showed that powder from untreated egg became acid more rapidly during storage. Reduction in the moisture content (total volatiles) from 4.7 to 3.0% doubled, and reduction from 4.7 to 1.7% tripled, the storage life of dried whole egg powder as assessed by fluorescence tests. The maximum storage life predicted for the low moisture powder by this test was only 36 wk. at 27° C. and 5 wk. at 38° C. Palatability tests suggested that the product was somewhat less perishable, as a powder of 1.7% moisture was considered fit for use as an egg dish after 64 wk. at 27° C. Gas-packing low moisture powders in an atmosphere of carbon dioxide appeared to be slightly more effective as a means of retaining palatability than packing in an atmosphere of air or nitrogen, but was particularly effective in preventing loss of solubility (assessed by potassium chloride values) during storage.

Introduction

During an investigation of the effect of added substances on the keeping quality of egg powder, it was observed that fluorescence development in powders containing either citric or lactic acid was more rapid than in control powder (3). Contrary indications had been observed elsewhere (4).

Accelerated tests, done in these laboratories, showed that the rate of deterioration of egg powder increased with increase in moisture content; therefore, it was recommended that, to maintain quality during storage and transport, dried egg should have a moisture content of not more than 5% and probably 2% or less (7). It was further noted that reduction to 1.4% had marked preservative action, although some deterioration occurred when powders were held at 37° and 48° C. (6).

A study of the effect on keeping quality of packing in nitrogen, carbon dioxide, *in vacuo*, and in the form of compressed tablets showed that only carbon dioxide had a beneficial effect (8). Continued investigation of the effect of carbon dioxide showed that this method of packing afforded some protection against heat deterioration, particularly on the solubility of the powder (6).

At the request of the Advisory Committee to the United States Army Quartermaster Corps, experiments were undertaken in co-operation with American research organizations to verify the advisability of combining those features believed to result in powders of better keeping quality, e.g.,

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acidifying liquid egg prior to drying, drying egg to low moisture content, and packing in carbon dioxide or nitrogen or both. The present paper deals with the storage behaviour of such specially treated powders, both at a relatively high temperature, 38° C. (100° F.), and a temperature likely to be met during ordinary handling of dried egg powders, 27° C. (80° F.).

Methods

The quality tests applied were: the evaluation of palatability by a taste panel of six persons scoring on a basis of 10 to 0, 10 being the equivalent of excellent fresh egg (1); a fluorescence measurement of an extract of defatted egg powder in 10% sodium chloride solution (2) and a measurement of solubility in a 10% potassium chloride solution (5). Measurements of pH were made on samples as reconstituted for cooking. Moisture content was determined as total volatiles by a modification of the standard A.O.A.C. vacuum oven procedure. The technique of this modification has been previously described (5).

Materials

The egg powders used in this investigation were prepared in a Canadian egg drying plant from frozen egg. One portion was dried by current methods, resulting in a product having a moisture and volatile content of 4.7%; another portion was dried to have the lowest moisture and volatiles feasible in a single stage commercial operation (3.0%); some of this powder was subjected to a second, combined cooling and vacuum drying process, which further reduced the moisture and volatile content to 1.7%. A further sample of liquid egg was acidified to a pH of 6.7 before subjection to drying and subsequent redrying to produce a low volatile powder (1.7%). These powders were stored in tin plate containers, the headspace gas being air.

Samples of the acidified and untreated products were also packed in tin plate in atmospheres of carbon dioxide and nitrogen. The carbon dioxide content of the headspace gas was approximately 100, 75, 50, 25, and 0%; the remainder of the gas was nitrogen except for a trace of oxygen.

Powders stored at 38° C. (100° F.) were sampled after 1, 2, 4, 8, and 16 weeks' storage, while powders stored at 27° C. (80° F.) were sampled after 4, 8, 16, 32, and 64 wk.

Results

The effect of moisture content is shown in Fig. 1, while effects of acidifying liquid egg and of using atmospheres of carbon dioxide and nitrogen, either alone or mixed, are given in Tables I and II and Fig. 2. Only the mean values for each variable, averaged over all other conditions, are shown in Tables I and II, since this was a convenient method of summarizing the data. The inert atmosphere in the headspace of the tins is recorded on an "as-packed" basis. Measurement after the one, two, and four weeks at 38° C.

and after four and eight weeks at 27° C. showed that the pressure inside the container had been reduced by about $\frac{1}{2}$ atm. and that the carbon dioxide content of the headspace gas was 90, 45, 5, 2, and 0%, the remainder of the gas being nitrogen and about 0.5% oxygen. Sorption of carbon dioxide by the powder was believed responsible for this phenomenon and is under investigation.

MOISTURE EFFECTS

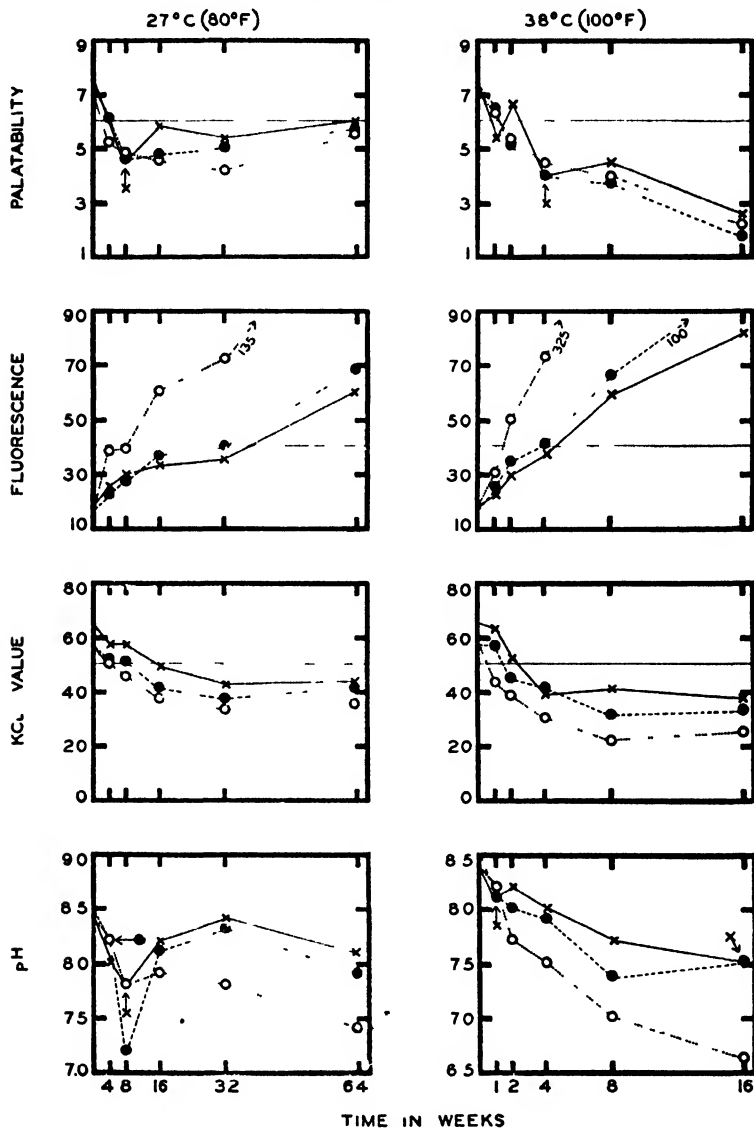


FIG. 1. The effect of moisture levels on the keeping quality of dried whole egg powder stored at 27° C. (80° F.) and 37° C. (100° F.). Light lines show limits of desirability as an egg dish. 4.7% moisture, ○; 3.0% moisture, □; 1.7% moisture, ●; 0.5% moisture, ×.

TABLE I

TABLES OF MEANS AND ANALYSES OF VARIANCE OF TESTS ON EGG POWDER OF 1.7% MOISTURE CONTENT PREPARED FROM LIQUID EGG WITH AND WITHOUT ADDED ACID AND STORED IN ATMOSPHERES OF CARBON DIOXIDE AND NITROGEN AT 38° C. (100° F.)

Tables of means

Variable under study	Palatability	Fluorescence value	Potassium chloride value	pH
<i>Powder</i>				
From acidified liquid egg	5.6	38.6	55.5	7.3
From untreated liquid egg	5.6	36.5	53.9	8.0
<i>Approximate gas composition¹</i>				
100% carbon dioxide	5.7	35.2	57.6	7.6
75% carbon dioxide	5.8	35.5	56.4	7.6
50% carbon dioxide	5.6	38.0	53.8	7.7
25% carbon dioxide	5.7	39.0	53.2	7.7
0% carbon dioxide	5.3	40.3	52.4	7.7
<i>Storage time</i>				
Initial	7.7	17.1	63.1	7.9
1 week	6.8	21.1	64.8	7.8
2 weeks	6.2	27.3	61.4	7.8
4 weeks	4.7	33.2	50.7	7.6
8 weeks	4.5	50.1	47.8	7.4
16 weeks	3.6	76.9	40.4	7.6

Analyses of variance

Variance attributable to:	Degrees of freedom	Mean square			
		Palatability	Fluorescence value	Potassium chloride value	pH
Powder (plain vs. acid)	1	0.00	66 **	35 *	7.9 **
Gas composition	4	0.40	58 **	61 **	0.026
Storage time	5	24.2 **	5035 **	968 **	0.452*
Powder × gas composition	4	0.06	5.6*	11	0.022
Powder × storage time	5	0.21	67 **	17 *	0.060*
Storage time × gas composition	20	0.35*	11.4**	7.3	0.026
Residual	20	0.15	2.0	4.8	0.020

¹ Remainder of gas, nitrogen.

* Exceeds the 5% level of statistical significance.

** Exceeds the 1% level of statistical significance.

Acid Powder

Acidifying liquid egg prior to drying appeared to afford little protection to the powder (Tables I and II). The only measurement showing significant differences between powders at both storage temperatures was, of course, pH. At 38° C., in addition to difference in initial pH, the powder prepared from untreated egg tended to become acid more rapidly than powder prepared from

TABLE II

TABLES OF MEANS AND ANALYSES OF VARIANCE OF TESTS ON EGG POWDER OF 1.7% MOISTURE CONTENT PREPARED FROM LIQUID EGG WITH AND WITHOUT ADDED ACID AND STORED IN ATMOSPHERES OF CARBON DIOXIDE AND NITROGEN AT 27° C. (80° F.)

Tables of means

Variable under study	Palatability	Fluorescence value	Potassium chloride value	pH
<i>Powder</i>				
From acidified liquid egg	6.2	27.6	57.0	7.4
From untreated liquid egg	6.3	28.4	56.8	8.2
<i>Approximate gas composition¹</i>				
100% carbon dioxide	6.3	27.2	59.3	7.8
75% carbon dioxide	6.4	26.4	58.2	7.8
50% carbon dioxide	5.9	27.7	56.6	7.8
25% carbon dioxide	6.3	27.8	55.6	7.8
0% carbon dioxide	6.0	30.9	54.8	7.8
<i>Storage time</i>				
Initial	7.7	17.1	63.1	7.9
4 weeks	5.4	22.2	60.5	7.8
8 weeks	5.6	23.2	62.4	7.6
16 weeks	5.7	29.5	55.8	7.8
32 weeks	6.5	29.4	51.7	8.1
64 weeks	6.3	46.6	47.9	7.6

Analyses of variance

Variance attributable to:	Degrees of freedom	Mean square			
		Palatability	Fluorescence value	Potassium chloride value	pH
Powder (plain vs. acid)	1	0.00	11	1.01	8.4 **
Gas composition	4	0.45	36 **	41 **	0.26 **
Storage time	5	7.4 **	1057 **	385 **	0.385**
Powder × gas composition	4	0.81	6.7	8.8	0.016
Powder × storage time	5	0.36	1.0	8.4	0.014
Storage time × gas composition	20	0.24	5.4	7.5	0.016*
Residual	20	0.32	3.7	5.3	0.006

¹ Remainder of gas, nitrogen.

* Exceeds 5% level of statistical significance.

** Exceeds 1% of statistical significance.

acidified liquid egg. While it is difficult to explain this phenomenon, it is believed of little practical importance since acidification of liquid egg prior to drying had no other significant effect.

Effect of Moisture Content

There is evidence from Fig. 1 that reduction in moisture content resulted in a slower rate of decrease in palatability during storage. Powder with a moisture content of 1.7% was about one palatability unit better than that

GAS - PACKING EFFECTS

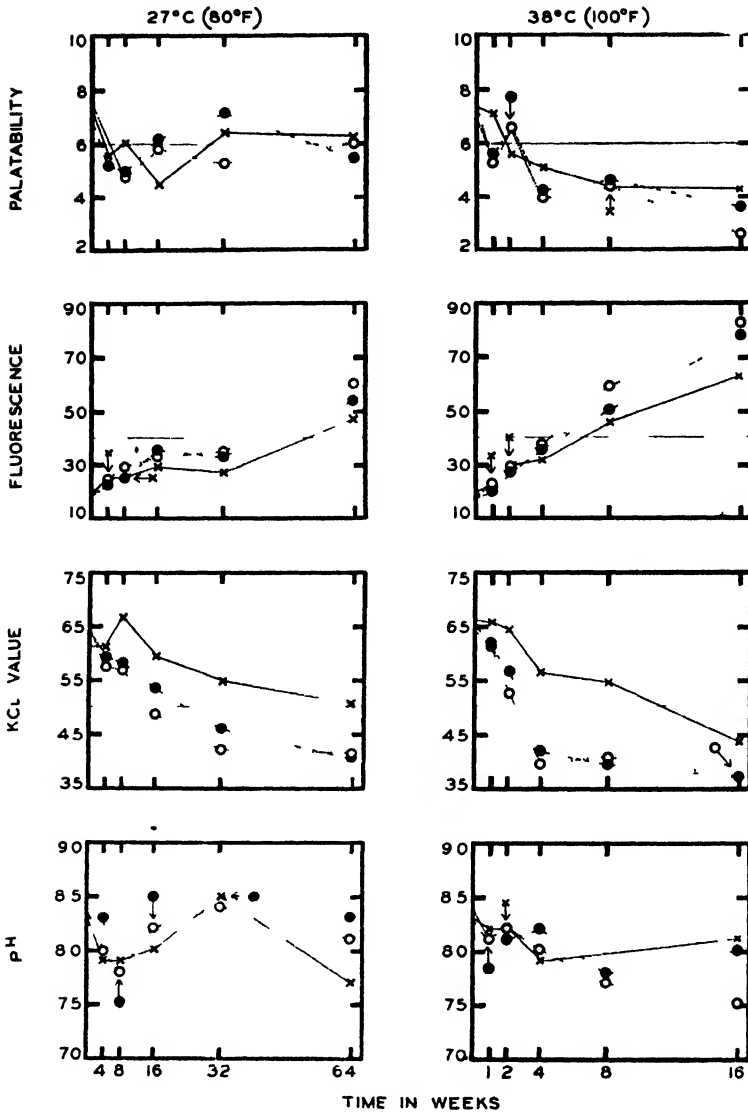


FIG. 2 The effect of gas-packing on the keeping quality of dried whole egg powder (moisture and volatiles 1.7%) stored at 27° C (80° F) and 37° C (100° F). Light lines show limits of desirability as an egg dish. Air, ○, nitrogen, ●, carbon dioxide, X.

with 4.7% moisture after storage for 16 wk at 38° C. and about half a palatability unit better after 64 wk. at 27° C. Powders stored at 27° C. for eight weeks had exceptionally low palatability and pH values; nevertheless the 1.7% moisture powder was still considered suitable as an egg dish after 64 weeks' storage in an atmosphere of air at 27° C.

It had been noted that fluorescence values of 40 are about the equivalent of a palatability score of 6, the limit of desirability of the powder as an egg dish (1). Using this criterion, it was observed that the reduction in moisture to 3% doubled, while further reduction to 1.7%, tripled the storage life at 38° C. (Fig. 1). However, even this extended life was only five weeks. At 27° C., powders having 4.7% moisture had a storage life of only about six weeks, while powders of 3.0 and 1.7% moisture withstood storage of 32 and 36 wk., respectively.

Measurements of potassium chloride value and pH, both related to palatability (5), also indicated an increase in storage life as volatile content was reduced.

Packing in Carbon Dioxide and Nitrogen

The effects of gas-packing egg powder of 1.7% volatile content in atmospheres of air, carbon dioxide, and nitrogen are shown in Fig. 2. The values depicted here contrast data from Fig. 1 with data from Tables I and II. Only the data for air, nitrogen, and carbon dioxide packing are shown since these showed most clearly the differences between methods of packing. Again palatability scores were variable, but there was some indication of increased storage life as a result of gas-packing, carbon dioxide being more effective than nitrogen, which was in turn more effective than air. Fluorescence and pH measurements also indicated that carbon dioxide was more effective than nitrogen in increasing storage life. The carbon dioxide packed material showed a marked drop in pH after 64 wk. at 27° C., thus explaining the significant differential effect noted in Table II. This may be the result of some reaction between carbon dioxide and egg powder to produce more highly acidic products.

Generally, slight improvement in palatability resulted from increasing the carbon dioxide content in the headspace gas (Tables I and II). Both fluorescence and potassium chloride values indicated that improvement resulted from increased carbon dioxide content. This improvement became more noticeable as storage progressed.

The most pronounced effect was evident in the improved solubility of the powders packed in an atmosphere of carbon dioxide: after eight weeks' storage at 38° C., powder in an atmosphere of carbon dioxide had a potassium chloride value of 55, while powders packed in air or nitrogen had a value of about 38. After 64 wk. at 27° C. the carbon dioxide packed material still had a solubility greater than 50.

Acknowledgments

The authors wish to thank Mr. D. B. W. Reid for making the statistical computations.

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THE EFFECT OF WHEAT GERM OIL ANTIOXIDANTS AND NATURAL REDUCING SUBSTANCES ON THE STABILITY OF WHOLE MILK POWDER¹

BY R. A. CHAPMAN² AND W. D. McFARLANE³

Abstract

Storage trials have been conducted on a large number of roller and spray-process whole milk powders. Accelerated tests at 65° C. were found to give an accurate indication of the relative keeping qualities of the samples. Wheat germ oil antioxidants were found effective in inhibiting deterioration due to copper. Reducing substances that develop in milk powders during storage in a moist atmosphere or at elevated temperatures are strong antioxidants and may offset the effect of added antioxidants. The riboflavin content of several powders with a high concentration of reducing groups decreased appreciably during storage.

Introduction

Tallowy odours and flavours produced by the action of atmospheric oxygen on the butterfat are considered to be one of the most important forms of spoilage in whole milk powder (13). Copper and iron contamination, due to the solvent action of heated milk on metallic surfaces during processing, is an important factor in the development of this defect. In recent years a number of investigations have been conducted in an effort to retard oxidative rancidity by the use of antioxidants.

Peters and Musher (17) claimed that the fresh flavour of powdered milk was retained when oat flour was added to liquid milk prior to spray-drying and when the oat flour was mixed with the dry powder. Waite (19) has used oat flour and hydroquinone with some success. However, the latter substance, at a level of 0.5% of the weight of the fat, imparted an objectionable metallic flavour to the milk powder. Jack and Henderson (9) have employed Avenex No. 7, Avenol, and ascorbic acid for this purpose. Avenex at a level of 2 to 3% of the milk solids, or 0.2% of Avenol, prevented the development of oxidized flavour for approximately eight months. Gum guaiac and hydroquinone were the most effective of the antioxidants employed by Hollender and Tracy (7), and ethyl gallate, at levels of 0.01 and 0.03% of the liquid milk, has been reported by Hilditch (6) to treble the life of spray-dried whole milk powders.

Lips and McFarlane (15) found that wheat germ oil fortified with citric acid was an effective antioxidant in the prevention of oxidative rancidity in lard and shortening. Since this combination is non-toxic and both constituents occur naturally in foods, it seemed desirable to investigate its use

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as an antioxidant in whole milk powder. A similar study by Tracy *et al.* (18), started at the same time as this investigation, has now been reported. They found that wheat germ oil was most effective at a level of 0.2% of the butter fat and the inhibiting effect was reinforced by citric acid.

Several workers (3, 11) have found that sulphydryl compounds, which developed in liquid milk heated to high temperatures, were responsible for the inhibition of oxidized flavours. Hollender and Tracy (7) reported that powders prepared from milk heated to 170° F. for 30 min. prior to drying was less likely to develop an oxidized flavour than those samples prepared from milk heated to 150° or 190° F. The presence of sulphydryl groups has been suggested by Jack and Henderson (9) as the reason for the improved stability of the product prepared from milk preheated to high temperatures. In view of these facts it appeared desirable to determine accurately the effect of these reducing groups on the stability of the butterfat in whole milk powders, especially in relation to the action of wheat germ oil antioxidants.

Early in this study it became evident that an accelerated storage test was an important requirement. Acceleration by light, heat, and metallic catalysts has been employed in the study of oxidative rancidity in fats and oils. Recently, however, Hilditch (6) has questioned the validity of all accelerated tests and heat-accelerated tests in particular. Heat-accelerated tests on milk powders have been discussed by Lea, Moran, and Smith (13) who found that temperatures above 60° C. rapidly produced caramel-like flavours and discoloration, while similar defects were obtained more slowly at 37° and 47° C. In this investigation these off-flavours masked the detection of oxidized flavours by organoleptic means, but had no apparent effect on the formation of peroxides.

Materials and Methods

A large number of milk powder samples containing the following antioxidants were prepared by commercial firms.

Formula A—Straight wheat germ oil.*

Formula B—Wheat germ oil plus 1% of citric acid.

Formula C—Wheat germ oil plus 2% of citric acid.

Formula D—65% wheat germ oil, 30% of soya-bean lecithin, 5% of citric acid.

Formulas B and C were prepared by dissolving citric acid monohydrate in absolute ethyl alcohol, thoroughly mixing the solution into the required amount of wheat germ oil, and agitating the mixture in a Waring Blendor for five minutes. The alcohol was then removed *in vacuo*. Formula D was prepared by the method outlined by Lips†.

It was noted that the peroxide value of wheat germ oil was frequently as high as 60 milliequivalents per kilogram. It was believed that if the peroxide

* The wheat germ oil employed throughout this investigation was the solvent extracted product prepared by Viobin Corp., Monticello, Ill., U.S.A.

† A. Lips, Ph.D. thesis, McGill University, Montreal, Que. 1944.

value of the oil were reduced, its stabilizing value would be enhanced. Therefore, the antioxidants were heated at 150° C. for 60 min. under vacuum in the presence of a nickel catalyst, since previous experiments had indicated that this treatment would remove the peroxides.

The treated antioxidants were then tested by the Swift stability test as modified by Lips and McFarlane (15). However, the results indicated that the treatment had not improved the stabilizing action of the oils. Vitamin E determinations by the method of Parker and McFarlane (16) revealed that the heat treatment had not reduced the tocopherol content.

The copper content of all the samples was determined by the method of Kerr (12) with minor modifications, which included the addition of a few drops of cresol red to indicate the presence of slight excess of ammonia before the addition of the carbamate reagent, and extraction of the coloured complex with carbon tetrachloride instead of chloroform. Solubility determinations were made according to the directions of Howat and associates (8). The milk powders were reconstituted at 60° C. as recommended by Lea, Moran, and Smith (13), cooled to 20° C., and centrifuged at 4000 r.p.m. The supernatant liquid was decanted into an evaporating dish and dried *in vacuo* at 100° C. for five hours.

Peroxides were determined by the method of Chapman and McFarlane (1). The procedure was found quite satisfactory but occasionally a reagent was prepared that developed a considerable amount of colour. This difficulty could be minimized if all the ammonium thiocyanate were dissolved before the addition of the anhydrous acetone (14). The solution was then thoroughly mixed before adding the ferrous ammonium sulphate. It was also necessary to purify the acetone carefully. Reducing groups were estimated by the method of Chapman and McFarlane (2).

Discussion of Experimental Data

Series I—Storage at Various Temperatures

In the early stages of the investigation, the milk powders were stored at room temperature and 37° C., but the deterioration was often relatively slow. It therefore was necessary to accelerate the oxidation of many samples by storing them at higher temperatures. An experiment was conducted to compare the stability of milk powders at room temperature and at elevated temperatures.

Two spray-dried samples were prepared, one of which contained Antioxidant *D* at a level of 0.1% of the butterfat. They were stored in open containers at room temperature, 37°, 55°, and 65° C. Peroxide values were determined at intervals with the results shown in Fig. 1.

The conclusion as to the relative stability of the two powders would be the same irrespective of whether the data obtained at 65° C. or at room temperature was considered. However, at 55° and 65° C. the samples developed caramel-like flavours, which tended to mask the tallowiness of the

butterfat as determined organoleptically. For our purpose the advantage of reducing the storage time from 150 to 15 days outweighed the disadvantage of not being able to test the quality of the sample by taste. The effect of the antioxidant was evident in all cases and the relatively poor keeping quality of the powder can probably be attributed to its high copper content (8.79 p.p.m.).

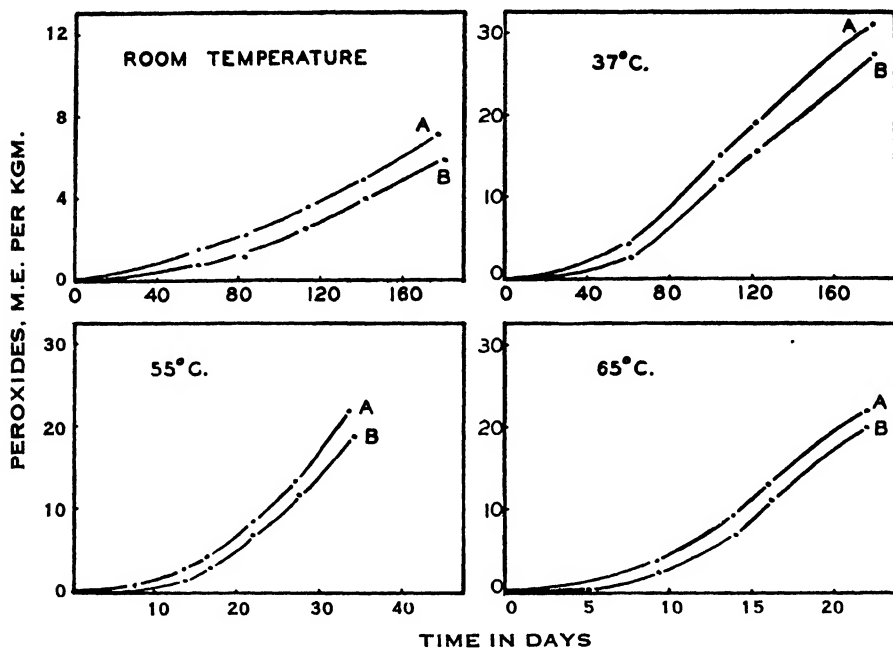


FIG. 1. The stability of spray-process whole milk powder as influenced by the storage temperature. Sample A—control, Sample B—treated with antioxidant D.

The results of another experiment, carried out to determine the effect of light in addition to heat, indicated that although light accelerated the oxidation of the butterfat, elaborate precautions would be necessary to ensure uniform exposure of the samples. Heat alone seemed more satisfactory as the basis of an accelerated test.

Series II—Storage at Room Temperature

Two samples of spray-dried whole milk powders were prepared with commercial equipment, and one was treated with Antioxidant D at a level of 0.1% of the butterfat. They were stored at room temperature in friction-top cans and sampled periodically. The results of the peroxide tests are shown in Fig. 2. Both powders were relatively unstable and this can be attributed chiefly to copper contamination, analyses showing 23.5 p.p.m. It is clear that under such conditions wheat germ oil D is a potent inhibitor of the catalytic action of copper.

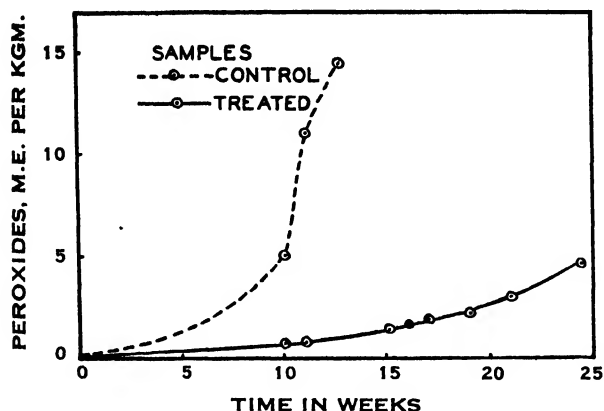


FIG. 2. The effect of a wheat germ oil antioxidant on the stability of spray-process whole milk powder stored at room temperature.

Series III

(a) Storage of Fresh Samples at 65° C.

A series of eight spray-dried powders, containing antioxidants *A*, *C*, and *D* at levels of 0.1 and 0.05% of the butterfat, was prepared and designated as follows:

- No. 24 – Control for 0.1% levels
- No. 26 – 0.1% wheat germ oil *A*
- No. 28 – 0.1% wheat germ oil *C*
- No. 30 – 0.1% wheat germ oil *D*
- No. 52 – Control for 0.05% levels
- No. 54 – 0.05% wheat germ oil *A*
- No. 56 – 0.05% wheat germ oil *C*
- No. 58 – 0.05% wheat germ oil *D*

One set of samples, packed in No. 1 tins, were opened on arrival from the manufacturing plant, reconstituted, and tested for flavour and odour. All samples were perfectly fresh and it was impossible to differentiate between the various treatments. A portion of each powder was then transferred to 125 ml. Erlenmeyer flasks, stored at 65° C., and tested periodically for peroxides. The results are given in Fig. 3. All the wheat germ oils indicated definite stabilizing ability, but antioxidant *D* at a level of 0.1% of the butterfat was slightly superior to the others.

(b) Storage at 65° C. after Period at Lower Temperatures

Portions of the fresh powders were also stored in open containers at room temperature and 37° C. After several months' storage it was evident that changes other than oxidative deterioration of the butterfat had occurred; the powders, especially those at room temperature, had developed stale, musty odours and flavours, yet no increase in peroxide value great enough to account for butterfat decomposition could be observed. Even at 37° C. the samples showed a very slow increase in peroxide values, as can be seen in Table I.

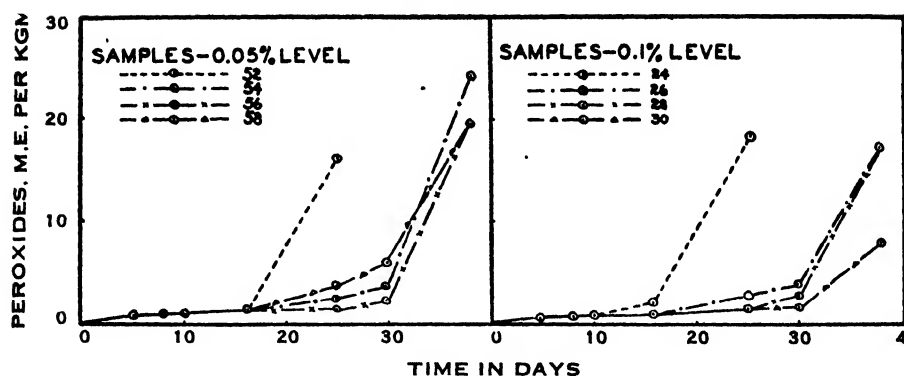


FIG. 3. Accelerated storage test at 65° C. on fresh samples of spray-process whole milk powders.

TABLE I

PEROXIDE VALUES OF MILK POWDERS STORED AT 37° C. EXPOSED TO THE AIR

Sample No.	Storage time, weeks						
	0	6	13	22	68	71	77
	Milliequivalents of peroxide per kgm. of milk powder						
24	1.8	2.3	2.0	1.6	0.9	2.3	3.4
26	1.1	2.5	1.1	0.5	2.5	3.6	4.1
28	1.4	2.3	1.4	0.7	5.2	10.0	12.7
30	1.3	2.5	2.5	0.7	0.5	0.9	3.1
52	0.9	0.5	2.0	0.7	0.2	1.4	0.9
54	0.8	0.7	1.4	0.5	3.2	6.1	7.0
56	0.8	0.9	2.2	0.5	0.5	5.0	7.7
58	0.6	0.5	0.9	0.6	2.2	1.1	1.1

Since these samples were not behaving in a manner similar to that of the samples in the original experiment (Fig. 3), an accelerated storage test at 65° C. was started and carried along with the experiment reported in Table I. Peroxides were determined periodically on these samples and the results, plotted in Fig. 4, show that the stabilities of the samples at 65° C. before and after storage were not related. Accelerated tests at 65° C. on the fresh powders (Fig. 3) indicated that the control samples, Nos. 24 and 52, deteriorated more rapidly than the samples containing antioxidants. However, after storage at 37° C., the results showed that samples Nos. 26, 28, 54, and 56 were the most readily oxidized. The milk powders, after storage at room temperatures, were very stable and even at 65° C. breakdown occurred only after 50 to 100 days.

To explain these anomalous results, the powders were analysed for moisture, solubility, and reducing groups. These values were compared with those for air-packed material stored for 17 months at room temperature in No. 1 tins. The results are presented in Tables II and III.

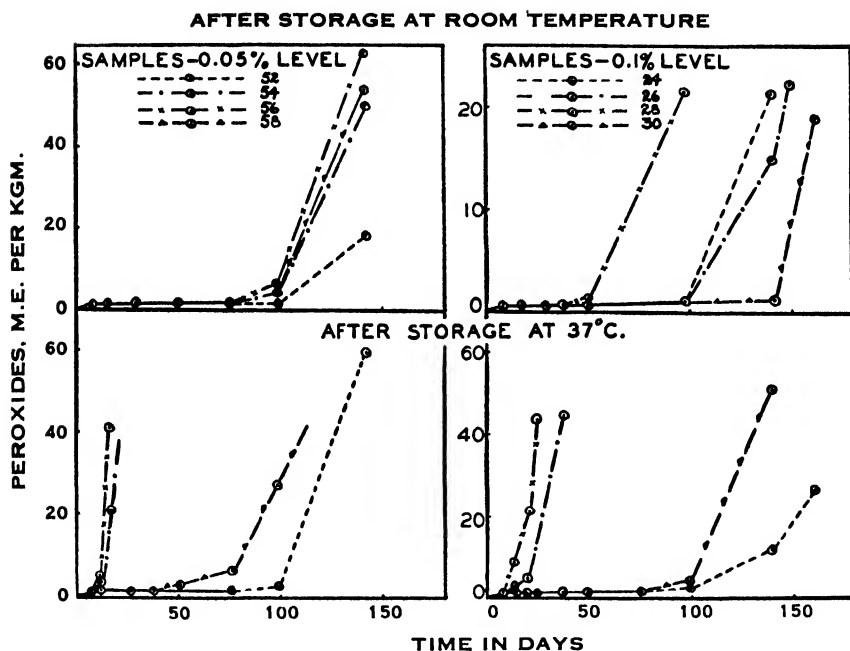


FIG. 4. Accelerated storage test at 65° C. on spray-process whole milk powder previously stored at room temperature and 37° C.

TABLE II

EFFECT OF STORAGE FOR 72 WEEKS ON AIR-PACKED MATERIAL IN NO. 1 TINS

Sample No.	Peroxide value	Reducing groups	Solubility, %	Moisture, %
24	4.3†	3.55*	92.5	2.20
26	2.7	3.75	92.4	3.08
28	2.5	3.75	91.9	3.74
30	2.5	3.85	92.1	3.90
52	3.8	3.55	92.3	3.74
54	2.3	3.65	92.3	3.74
56	3.0	3.20	92.9	2.54
58	4.1	4.40	92.0	2.62

† Milliequivalents of peroxide per kilogram of milk powder.

* Moles $\times 10^5$ reducing groups per gram of milk powder.

These results provide an explanation of the fact that the relative stability of the milk powders was now reversed. The solubility of the samples stored at room temperature had decreased more than that of the samples stored at 37° C. and the moisture content was apparently a factor. The higher values obtained in August 1943, for the moisture content of the samples at room temperature, were probably due to a higher relative humidity in the laboratory than in the 37° C. oven. The increased moisture content had more than offset the denaturing effect of the higher temperature.

TABLE III
EFFECT OF STORAGE AT ROOM TEMPERATURE AND AT 37° C.

Sample No.	Reducing groups	Solubility, %	Moisture	
			Aug. 1943	April 1944
<i>Stored at room temperature</i>				
24	20.3*	45.0	5.21	3.09
26	22.0	45.5	—	3.07
28	21.0	44.8	—	3.18
30	21.0	51.1	4.65	3.32
52	22.1	48.0	4.66	2.84
54	20.7	47.8	—	3.30
56	18.0	46.3	—	3.39
58	22.7	50.6	4.53	3.12
<i>Stored at 37° C.</i>				
24	17.5	56.2	4.23	3.08
26	14.4	70.1	—	3.23
28	14.5	68.4	—	3.44
30	19.9	48.5	4.02	2.67
52	17.8	60.1	4.10	2.96
54	15.2	81.0	—	3.40
56	15.2	82.5	—	3.35
58	17.2	67.9	3.94	3.03

* Moles $\times 10^5$ reducing groups per gram of milk powder.

The quantitative changes in the reducing groups of the milk powders were pertinent in explaining the relative stability of the samples. From an initial value of approximately 3.75×10^{-5} moles of reducing groups per gram, the powders open to the air attained values of 22.7×10^{-5} moles, with the room temperature samples showing consistently higher values. Of the samples stored at 37° C., Nos. 26, 28, 54, and 56 were lower in reducing groups than either the control or the sample with wheat germ oil *D*. They also had the shortest induction period in the accelerated test and the highest values for solubility.

Series IV—Storage Trials at 65° C. after Preliminary Storage at Room Temperature

The whole milk powders in this series were prepared with standard spray-drying equipment and were identified as follows:

Control – No antioxidant

A – 0.75% wheat germ oil *A*

AA – 1.50% wheat germ oil *A*

C – 0.75% wheat germ oil *C*

CC – 1.50% wheat germ oil *C*

D – 0.75% wheat germ oil *D*

DD – 1.50% wheat germ oil *D*

The powders were stored in fibre containers and sampled periodically for the determination of peroxides. As there was no appreciable increase in peroxide values after eight months' storage, portions were removed and subjected to an accelerated test, the results of which are shown in Fig. 5. The

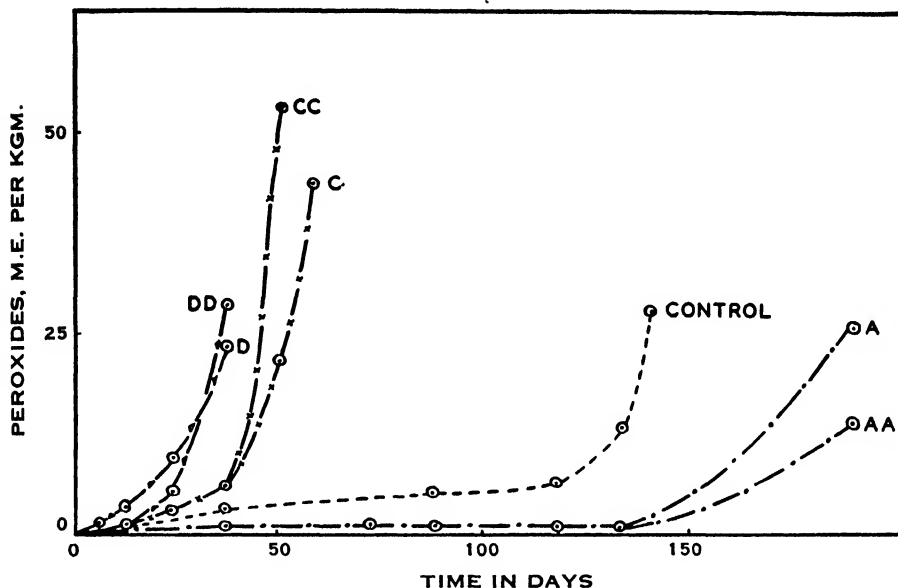


FIG. 5. Accelerated test at 65° C. on spray-process whole milk powders previously stored at room temperature.

samples containing antioxidant *A* were more stable than the control but the milk powders treated with antioxidants *C* and *D* deteriorated more rapidly. Reducing groups and solubilities were then determined in an attempt to ascertain the cause of this variation. The results are given in Table IV.

The samples treated with antioxidants *C* and *D* gave the lowest values for reducing groups and were the least stable. The control and powders *A* and

TABLE IV
REDUCING GROUPS AND SOLUBILITY OF POWDERS

Sample	Reducing groups	Solubility, %
Control	21.2*	57.7
<i>A</i>	7.1	92.6
<i>AA</i>	13.4	85.9
<i>C</i>	5.25	94.1
<i>CC</i>	5.30	93.8
<i>D</i>	3.25	93.9
<i>DD</i>	5.55	94.9

* Moles $\times 10^4$ reducing groups per gram of milk powder.

AA showed a much longer induction period and also had a higher content of reducing substances. These findings were not in complete agreement with the results of similar accelerated tests reported in Series III, where the milk powders containing antioxidants *A* and *C* were the least stable. They show a disparity in the behaviour of a given antioxidant but the relation between reducing groups and stability is confirmed. Copper contamination was not appreciable, analyses of two samples showing only 1.75 and 1.81 p.p.m.

Series V—Storage Trials at 65° C.—Roller-process Whole Milk Powders

This experiment was carried out with roller-process whole milk powders containing wheat germ oil antioxidants as follows:

Control — No antioxidants

A — 0.1% wheat germ oil *B*

B — 0.1% wheat germ oil *A*

C — 0.1% wheat germ oil *C*

These samples were stored in friction-top cans at room temperature and were examined periodically. As there was no appreciable increase in peroxide values after 18 months' storage, a portion of each sample was subjected to an accelerated test at 65° C., the results of which are shown in Fig. 6.

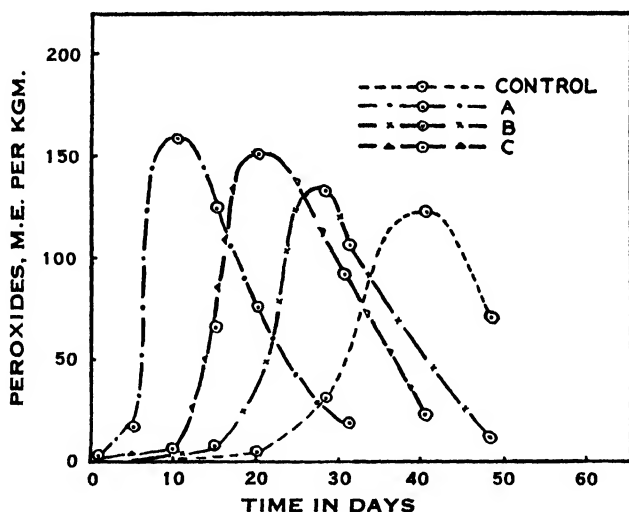


FIG. 6. Accelerated test at 65° C. on roller-process whole milk powders previously stored at room temperature.

The wheat germ oil actually appeared to behave as a pro-oxidant, in Samples *A*, *B*, and *C*. This experiment also showed clearly that a single peroxide determination could fail to indicate the quality of a milk powder since the peroxide values pass through a maximum. Peroxide and reducing values obtained after 27 months' storage at room temperature are given in Table V.

Sample *A* was definitely tallowy and although the others were stale they were not rancid. These roller-dried powders had a high content of reducing

substances, owing no doubt to the high temperature of processing. The presence of such antioxygenic substances probably accounts for the much longer storage life of this type of powder. The peroxide values indicated

TABLE V
REDUCING GROUPS AND PEROXIDE VALUES AFTER
27 MONTHS' STORAGE

Sample	Reducing groups	Peroxide values
Control	27.4*	0.7†
A	25.9	23.6
B	27.7	0.9
C	25.9	0.9

* Moles $\times 10^5$ reducing groups per gram of milk powder.

† Milliequivalents per kilogram of milk powder.

that the samples were deteriorating in the same order as shown in the accelerated test. The average copper content of these powders was 3.75 p.p.m.

THE EFFECT OF HEAT AND MOISTURE ON THE DEVELOPMENT OF REDUCING GROUPS

It is known that the solubility of milk powders with a high moisture content rapidly decreases during storage and that this effect is accompanied by the development of stale, musty odours and flavours. An experiment was therefore carried out to ascertain the effect of high moisture content on reducing groups and peroxide values.

Milk powders were held at 75% relative humidity by placing them in a large desiccator containing glycerol solution of sp. gr. 1.121 (10). Reducing groups, peroxide values and solubilities were determined at intervals, with the results shown in Tables VI and VII. The effect of storage under these conditions was to decrease the solubility and peroxide value and increase the content of reducing substances.

TABLE VI
EFFECT OF STORAGE IN A HUMID ATMOSPHERE ON THE REDUCING GROUPS

Sample No.	0 days	11 days	20 days	36 days
24	3.55*	4.95	8.45	11.80
26	3.75	5.25	8.15	11.55
28	3.75	4.95	9.10	12.50
30	3.85	5.10	8.25	11.80
61	6.35	6.50	10.35	11.95
63	6.60	7.40	10.20	11.75

* Moles $\times 10^5$ reducing groups per gram of milk powder.

TABLE VII

THE EFFECT OF STORAGE UNDER HUMID CONDITIONS ON MOISTURE,
PEROXIDE VALUE, AND SOLUBILITY

Sample No.	Solubility, %		Peroxide		Moisture, %	
	Original value	After storage	Original value	After storage	Original value	After storage
24	92.5 ¹	44.9	4.3†	0.7	2.20	9.02
26	92.4	45.1	2.7	0.4	3.80	8.95
28	91.9	45.3	2.5	0.7	3.74	9.29
30	92.1	44.7	2.5	0.2	3.90	7.54
61	70.0	46.6	1.3	0.2	3.06	8.61
63	67.5	45.1	1.0	0.4	3.05	9.47

¹ These values have been reported previously but are included here for comparison.

† Milliequivalents of peroxide per kilogram of milk powder.

Evidently the higher concentration of reducing substances was responsible for the decrease in peroxide values. These samples finally developed stale flavours and a very definite "cheesey" aroma, which was probably due to micro-organisms. The effect of heat was ascertained by determining reducing groups in these same samples during an accelerated test at 65° C. The results in Table VIII show that there was a relatively rapid increase in reducing groups during the initial period of storage, followed by a more gradual increase.

TABLE VIII

THE EFFECT OF STORAGE AT 65° C. ON THE REDUCING GROUPS

Sample No.	Reducing groups ¹		
	12 days	21 days	33 days
24	11.6*	13.7	13.5
26	12.2	14.3	13.9
28	10.5	13.5	13.6
30	13.1	13.5	14.2
61	14.0	17.5	19.1
63	14.3	16.9	18.7

¹ Original values of these powders are given in Table VI.

* Moles $\times 10^5$ reducing groups per gram of milk powder.

THE EFFECT OF REDUCING GROUPS ON THE RIBOFLAVIN CONTENT

Since riboflavin is readily destroyed by reduction, it seemed probable that the presence of reducing groups might have some effect on the vitamin B₂ content of the milk powder. A number of analyses were therefore made on representative samples by the method of Hand (4) adapted for use with milk powder.

The only apparent effect of a high concentration of reducing substances was noted in Series III. The samples that had been stored open to the air, at room temperature, had an average riboflavin content of 11.6 $\mu\text{gm. per gm.}$ The milk powders stored at 37° C. and at room temperature in sealed containers averaged 13.2 and 13.3 $\mu\text{gm. per gm.,}$ respectively. The results shown in Tables II and III indicate that the concentration of the reducing groups was much higher in the samples in which there was a definite decrease in riboflavin content. It therefore appeared probable that these reducing substances were responsible for the destruction of the vitamin.

Discussion and Conclusions

Accelerated tests at 65° C. give an accurate indication of the relative keeping qualities of whole milk powders. Only chemical tests can be used under such conditions because caramel-like flavours interfere with organoleptic examination. Samples of wheat germ oil had a considerable peroxide content but the removal of the peroxides did not improve the antioxygenic properties of the oil. Wheat germ oil alone and in combination with citric acid and lecithin was effective in inhibiting oxidation catalyzed by copper contamination. In powders of normal copper content, the increase in reducing substances that occurred during prolonged storage open to the air offset the effect of the antioxidants.

There was a rapid increase in reducing substances in milk powders stored in a humid atmosphere or at elevated temperatures. The relative stability of samples in a particular series was related to their content of reducing substances. The concentration was found to be much higher in roller-dried powders than in spray-dried samples, probably owing to the higher temperatures employed in the former process. This may be responsible for the greater stability of roller-dried products. The peroxide content was of value in following the progress of oxidative deterioration but it was also necessary to determine the concentration of reducing groups since these were likewise associated with off-flavours.

Harland and Ashworth (5) have recently used thiamin disulphide for the estimation of reducing substances in processed milk. They state that certain reducing compounds are liberated from liquid whole-milk and skim-milk powder at temperatures above 70° C. but if the heating were continued for 10 min. at 80° C., or at higher temperatures, the concentration decreased. These findings are contrary to the results obtained in this laboratory by the ferricyanide method (2). We have found that reducing substances are present in raw milk, and the amount increases on heating at temperatures up to 100° C. In addition, heated skim-milk powder and roller-process whole milk powder contained a much higher concentration of reducing substances than the original milk. Evidently the reagents are estimating different types of reducing compounds. In this investigation the storage tests were continued beyond the stage at which the samples were palatable because it appeared of

value to determine the factors that were responsible for the chemical changes in the advanced stages of deterioration.

Acknowledgments

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DRIED MILK POWDER

IV. THE EFFECT OF STORAGE TEMPERATURE, MOISTURE CONTENT, AND PLANT SOURCE ON THE KEEPING QUALITY OF MILK POWDERS OF DIFFERENT FAT LEVELS¹

BY W. A. BRYCE² AND J. A. PEARCE²

Abstract

Milk powders with fat contents of 1, 26, 28, and 30% from two plants were tempered to moisture contents of 2, 3, and 5% and stored for periods up to 16 weeks at temperatures of from 40° to 140° F. Appreciable deterioration, assessed by palatability, occurred in the whole milk powders stored at temperatures of 60° F. and higher, and there was considerable difference in the stability of powders from the two plants. For both plants, the keeping quality of powders of 26 and 28% of butter fat was equal. At 80° F. and lower, the powder containing 30% of butter fat was more stable than the 26 and 28% powders from the same plant, but at higher temperatures the 30% powder deteriorated more rapidly. At 80° F. the average decrease in palatability of whole milk powders with 2% moisture was two palatability units. The palatability of the skim-milk powder increased greatly at all temperatures during the early part of the storage period, but later decreased at temperatures of from 100° to 140° F. Skim-milk powder of 2% moisture stored at 80° F. had a palatability score 2.5 units higher than the initial score. In general, a moisture content of 3% was preferable to moisture contents of 2 and 5% for both whole and skim-milk powders. The differences in stability of powders from different plants were enhanced by increased moisture contents and higher storage temperatures.

Introduction

Experience gained during the war years has emphasized that improved methods of handling and storing must be developed if dehydrated foods are to assume their proper place in the post-war period. The importance of dried milk in the national diet has focused considerable attention on studies of production methods and keeping quality of this material.

Studies on the effect of temperature on the development of storage flavours in dried milk have yielded conclusions that have been somewhat conflicting. In one investigation (1) little difference was observed between powders stored at 40° F. and at 68° F., but a marked difference at 100° F. was reported. Powders from partially skimmed milk did not develop tallowiness when stored for 18 months at 32° F., but at 68° F. this off-flavour was noticeable in from five to six months. The rate of deterioration of whole milk powders has been found to increase rapidly at temperatures above 32° F. (4). It has also been reported (9) that the palatability decreased more rapidly at 117° F. than at 100° F. A storage temperature of 100° F. has been found to be better than either 80° or 120° F. (7).

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The effect of moisture on the keeping quality of both spray and drum-dried powders has been studied by a number of investigators (1, 2, 4, 9) who have found that moisture contents below 2% accelerated the development of tallowiness in spray-dried powders, and that at moisture contents above 5%, deterioration occurred rapidly in powders of all types. Little difference was observed in the effect of moisture contents between 2.3 and 5.4% on the development of tallowy flavours in powders of 30% butter fat, but with powders of 28% fat, a moisture content of 3.5% was preferable to either 2 or 5%. In general it has been found that the optimum moisture content required to reduce the development of storage flavours to a minimum is between 2 and 3%. It has been recognized that the keeping quality of dried milk is a function of both storage temperature and moisture content, increased moisture contents accelerating the effect of high storage temperatures.

It is evident that some disagreement exists in the published data on the keeping quality of milk powders. This paper discusses an investigation designed to evaluate the effects of moisture content and storage temperature on keeping quality, and at the same time to consider the effects introduced by different processors, and by the use of powders of different fat levels.

Materials and Methods

The powders used were commercial spray-dried products made by two Canadian companies from milk produced in the spring of the year. Powders of 1, 26, and 28% of fat were supplied by one plant, and of 1, 26, 28, and 30% of fat by another.

Each type of milk powder was divided into three portions, one of which was tempered to a moisture content of 2% by vacuum desiccation over phosphorus pentoxide, and was stored in tin plate (air as headspace gas) at temperatures of 40°, 60°, 80°, 100°, 120°, and 140° F. (4.4°, 15.6°, 26.8°, 37.8°, 49°, and 60° C.). The moisture contents of other portions were adjusted to 3 and 5%, respectively, by exposure to an atmosphere of high humidity, and were stored in a manner similar to those at 2% moisture, but at temperatures of 80°, 100°, and 120° F. only.

Palatability was the only quality test used that was satisfactory (7). The powders were tested for quality both initially and after storage for 2, 4, 8, and 16 weeks by an organoleptic method previously reported (7). The reconstituted milk was scored by a panel of 14 tasters and given a rating of from 10 to zero, 10 being the equivalent of best quality fresh whole or skim-milk.

Results

Effect of Temperature

The palatability results were analysed by statistical methods. The significant effects of different temperatures on each of the whole milk powders of 2% moisture are shown graphically in Fig. 1, and a summary showing averages for all products stored at the different temperatures is presented in

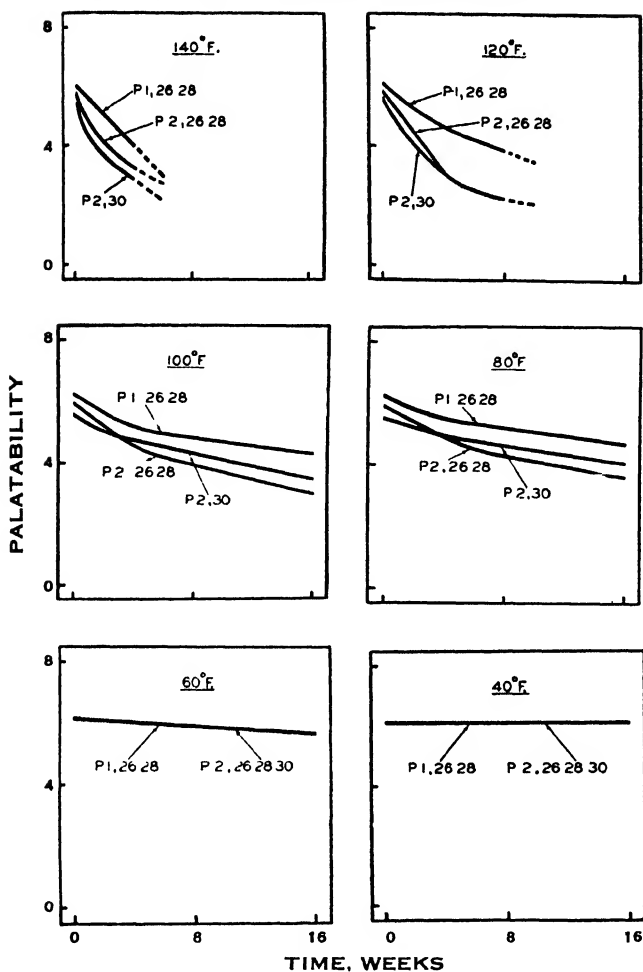


FIG. 1. The effect of storage temperature on the palatability of whole milk powders of different fat levels from Plants 1 and 2. All samples at 2% moisture.

Fig. 2. Although there was an appreciable difference between powders from the two plants, the powders of 26 and 28% butter fat from either of the plants behaved in a comparable manner at each storage temperature.

At the higher temperatures of 80° to 140° F., the material from Plant 1 was given a consistently higher palatability score than that of comparable fat content from Plant 2, showing that the product from Plant 2 was less stable than that from Plant 1. At the lower temperatures, the behaviour of all powders was the same. At 80° and 100° F., the 30% butter fat powder was given a higher palatability rating than the 26 and 28% powders from the same plant. At 120° and 140° F. the 30% powder was scored lower than the 26 and 28% powders.

Storage for 16 weeks at temperatures of 60° F. and lower had little effect on the palatability of all whole milk powders investigated, although a slight

downward trend was observed at 60° F. Typical storage flavours developed in powders at temperatures of 80° F. and higher, the rate of deterioration increasing with temperature. The data in Fig. 1 do not agree with observations previously reported (6, 7, 10) that milk powder maintained a higher palatability when stored at 100° F. than at either 80° or 120° F.

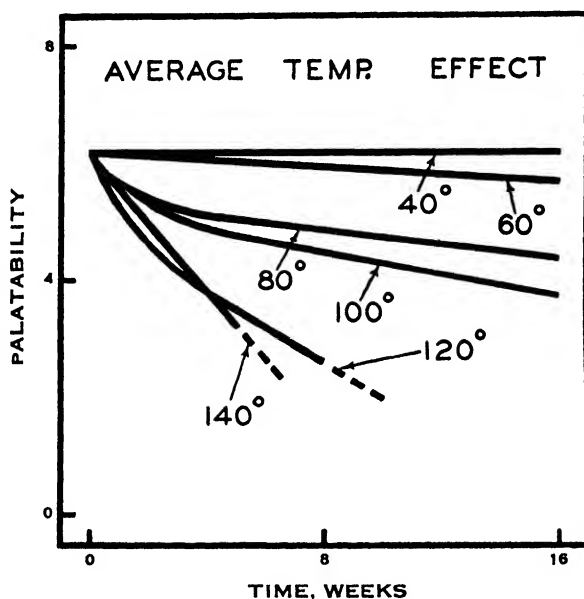


FIG. 2. The effect of storage temperature on the palatability of whole milk powders (averages of all samples) at 2% moisture.

The existence of an induction period in the deterioration of milk fat has been reported (5), but the present data gave no indication of such a phenomenon when palatability was used as a quality test. It can be seen from Fig. 1 that the rate of deterioration of all whole milk powders at all temperatures approximated a straight line relation, although the decrease in the rate of deterioration previously observed (7, 10) was noticeable. If an induction period existed, it must have come within the first two weeks of storage.

A comparison of the deterioration in skim-milk powder from the two plants is shown graphically in Fig. 3, and a summary of the temperature effects is presented in Fig. 4. Considerable difference was observed in both the initial palatability and the changes occurring during storage. The data for 40° and 60° F. have been combined as there was no real difference between the values for the powders at these two temperatures.

In contrast to the results obtained from the study of whole milk powders, it was found that the skim-milk powder from Plant 2 was much superior in keeping quality to that from Plant 1. However, throughout the experiment, the over-all behaviour of the two skim-milk powders was almost parallel at all temperatures. Under all conditions both products exhibited an initial

increase in palatability over the first four weeks of storage. At temperatures of 140°, 120°, and 100° F. the trend was downward after this initial increase was past, but below 100° F. there was no appreciable change during the next 12 weeks. The average palatability of both powders rose to a higher value as the temperature decreased (Fig. 4).

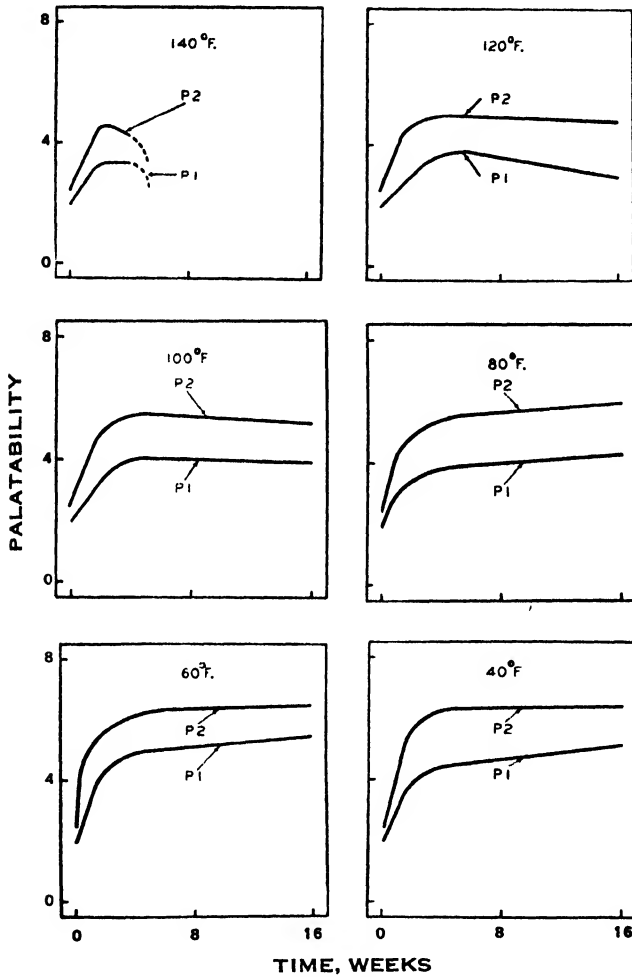


FIG. 3. The effect of storage temperature on the palatability of skim-milk powders from Plants 1 and 2. All samples at 2% moisture.

Effect of Moisture

The significant effects of moisture content on the keeping quality of dried whole milk powders are shown in Fig. 5. As in Fig. 1, the data for powders of 26 and 28% butter fat are combined, as the behaviour of these powders was identical. The different behaviour of the 30% butter fat powder was observed at all moisture levels. At 80° F. the 30% powder was given a

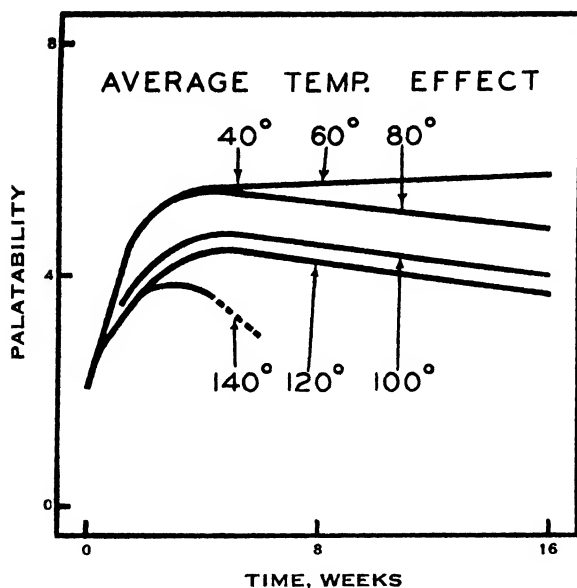


FIG. 4. The effect of storage temperature on the palatability of skim-milk powders (averages of all samples) at 2% moisture.

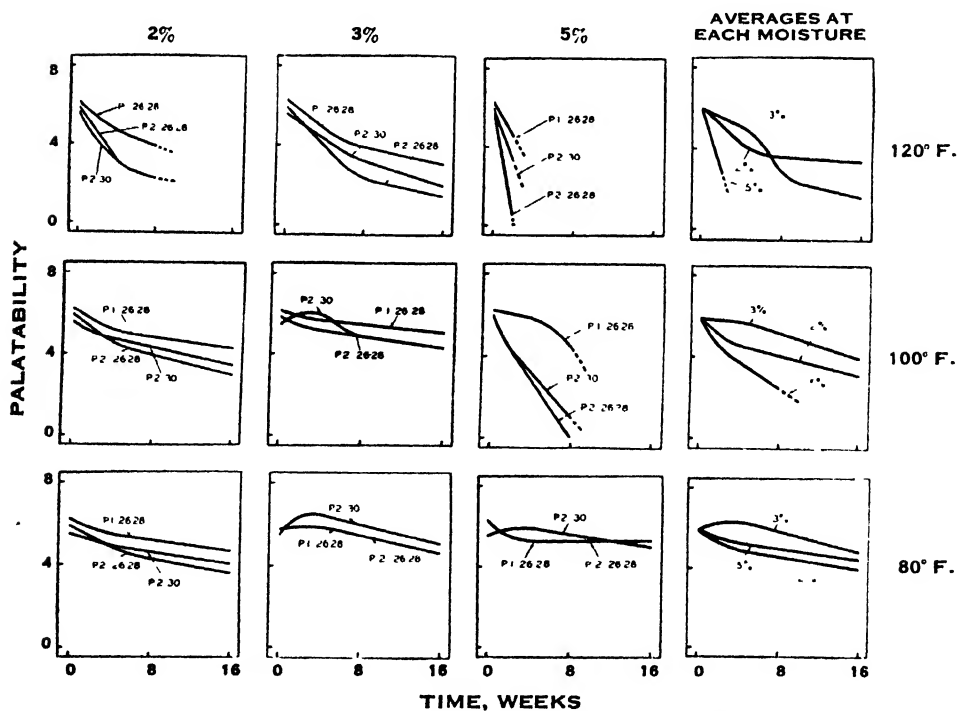


FIG. 5. The effect of moisture content and storage temperature on the palatability of whole milk powders from Plants 1 and 2.

slightly higher palatability rating than the 26 and 28% fat powders from the same plant. For all moisture levels, at higher temperatures, the difference in origin of the powders introduced greater variations than did the difference in fat content. The variation in stability of the powder with plant source was enhanced by increased moisture contents and by higher storage temperatures. A comparison of powders of equal fat levels at 100° and 120° F. showed that the keeping quality of powder from Plant 1 was superior to that of the powder from Plant 2 at all moisture levels.

The effect of moisture on the keeping quality of the powders was marked. At 120° F. the palatability of the powders of 2 and 3% moisture decreased at comparable rates, but those at 5% moisture deteriorated very rapidly. Considerable browning of these latter powders was observed after only two weeks of storage, and the palatability had decreased to such an extent that no further tasting was done. At 100° F. the powders with 3% moisture were markedly superior to those with moisture contents of 2 and 5%. At 80° F. the quality of the 3% moisture powders was appreciably better than that of the 2 and 5% powders although the differences were not as great as they were at 100° and 120° F. Only at 80° F. did the quality of the powders of 5% moisture remain higher than that of the powders of 2% moisture.

The combined effect of moisture content and storage temperature on keeping quality of whole milk powder can readily be seen from the graphs in Fig. 5, which show averages for all powders at each moisture level. At temperatures of 80° and 100° F., the superiority of the 3% moisture powder was evident. This was also evident during the first eight weeks of storage at 120°, but by the end of the sixteenth week the palatability of the 3% moisture powder had dropped below that of the 2% powder. Both the rate of deterioration shown by the slope of the curve and the actual palatability level of the powder were dependent on moisture content and storage temperature.

The data for the study of moisture effects on the keeping quality of skim-milk powders are shown graphically in Fig. 6. The superiority of the powder from Plant 2 over that from Plant 1 was observed for all moisture levels. The palatability change in the two products was about the same, the principal difference between them being in the initial quality.

As shown by the graphs of average palatabilities at each moisture level, the powders at a moisture content of 3% were again found to be superior to those at 2 and 5%, although the difference between the 2 and 3% powders was not marked. Storage life of the powder containing 5% moisture was considerably shortened at 120° and 100° F. but at 80° F. this powder was scored as high as were the 2 and 3% powders.

The marked initial increase in palatability exhibited by the skim-milk powder with a 2% moisture content was also observed for the 3 and 5% powders. At 80° F., this increase continued in powders of 2 and 5% moisture throughout the storage period. The level to which the palatability of the 5% powders rose was found to decrease with increasing temperatures.

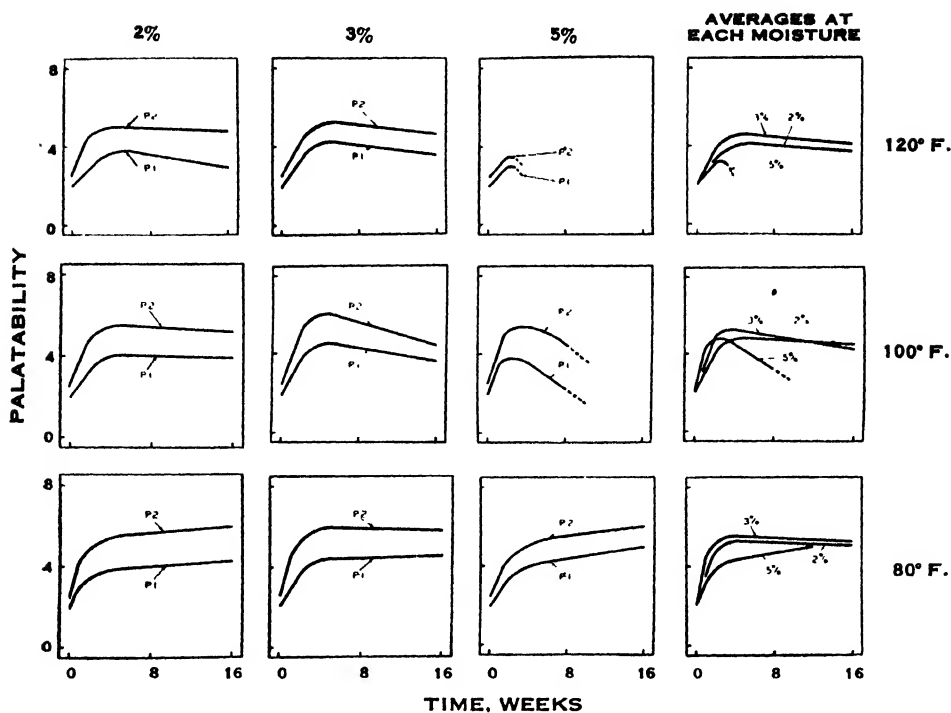


FIG. 6. The effect of moisture content and storage temperature on the palatability of skim-milk powders from Plants 1 and 2.

Discussion

This work has shown that considerable difference existed between plants in the effect of temperature on the keeping quality of whole milk powders. Deterioration was measurable at temperatures of 60° F., the rate of deterioration increasing with temperature and approximating a straight line relation. For skim-milk powders the marked difference in initial palatability between plants was uniformly maintained throughout the storage period. For both plants at all temperatures the palatability of skim-milk powders increased during the early part of the storage period.

Both whole and skim-milk powders at a moisture content of 3% maintained a higher palatability under storage than did these powders at moisture contents of 2 or 5%. Increased moisture contents were found to enhance the difference existing in the stability of powders from different plants.

The increase in palatability with storage of skim-milk powders has not been satisfactorily explained. It may be the result of the recombination in the powders of materials formed during early processing that was responsible for the low initial palatability. Protein degradation products such as various amines and dipeptides may recombine to form polypeptides whose flavours are not objectionable. At 80° F. this recombination may be sufficient to off-set the normal deterioration due to storage, and hence at this temperature

the palatability continues to increase throughout the entire storage period. Another explanation is that the concentration of these undesirable products may have been reduced by volatilization during storage (8). Further study of this problem is being made at the present time.

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THE KEEPING QUALITY OF DEHYDRATED MIXTURES OF EGG AND MILK¹

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Abstract

The storage life of a dehydrated mixture of egg and milk, when assessed by both palatability and fluorescence measurements, was shorter than the life of milk powder of similar protein, fat, and carbohydrate content. Increased quantities of egg in the mixture decreased the quality of the mixture, both initially and during 16 weeks' storage. These effects were noticeable at all temperatures studied between 40° and 140° F. but were most marked above 80° F. After 16 weeks at 80° F., material packed under carbon dioxide usually had better palatability than the air-packed products. The effect of added sugar was most noticeable at 120° and 140° F. Lactose had a slightly beneficial effect; sucrose was more effective.

Introduction

Preliminary data obtained in these laboratories indicated that dehydrated mixtures of milk and egg were less stable during storage than were either of these components separately. This becomes a matter of importance if these dehydrated mixtures are to be prepared for satisfactory reconstitution into ice cream, custard mixes, milk shakes, or other similar foods. Mixes such as these are commercially available in Canada and are also being used to prepare ice cream for the American armed services (12). Possible use by the Canadian armed forces led to a study of their keeping quality.

The addition of sugar to milk before dehydration was believed to improve the keeping quality of the product (4) and the addition of sucrose to eggs before drying retarded fluorescence development (11), which in turn is related to egg powder quality (9). Therefore, the preservative effect of sucrose and lactose on these materials was evaluated, since products of the type described are usually used in the preparation of sweetened dishes and the addition of a small amount of sugar to the liquid before drying would not affect its use.

Packing milk powder under an atmosphere of carbon dioxide was reported to extend the storage life of this product (5), although recent evidence indicated that any beneficial effect was not evident to a taste panel (14). Egg powder when packed under this gas deteriorated less rapidly than air-packed material (8). It was desirable in this study to obtain additional information about the effect of gas-packing by comparing carbon-dioxide-packed with air-packed mixtures.

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Materials and Methods

Mixtures Used and Storage Conditions

The materials used were obtained from commercial Canadian sources. Some formulas were prepared by mixing, and dehydrating, using a laboratory spray drier (15); others, by mixing the ingredients in the dry state. Differences in moisture content of dried egg powder (13) and dried milk powder (3) affect the keeping quality of both of these products. Therefore, the moisture content of the mixtures was kept within a range usually satisfactory for both dehydrated egg and milk (2 to 4%).

TABLE I
CALCULATED FAT, PROTEIN, AND CARBOHYDRATE PERCENTAGES OF VARIOUS
SPRAY-DRIED EGG AND MILK MIXTURES
(Products at 2.5% moisture)

Mixture	Fat	Protein	Carbo- hydrate	Ash
(A) 2.4 lb. of skim-milk powder in 20 lb. of fresh whole milk	15	31	47	4.8
(B) 1 doz. fresh eggs, 2.4 lb. of skim-milk powder, in 20 lb. of fresh whole milk	20	35	38	4.4
(C) 5 doz. fresh eggs, 2.4 lb. of skim-milk powder in 20 lb. of fresh whole milk	30	42	22	4.0
(D) 1 lb. of lactose, 5 lb. of fresh eggs, 1 lb. of skim-milk powder in 15 lb. of fresh whole milk	21	30	41	4.5
(E) Dry mix of whole milk powder, whole egg powder, dried fermented egg albumen, and lactose in proportions of 147: 78: 76: 125	17	33	45	3.5
(F) Dry mix of whole milk powders, skim-powder and whole egg powder in proportions of 147: 200: 78	17	35	39	6.4

The formulas investigated and the calculated analyses are given in Table I. Formula *A* gave a mixture without egg; *B* gave a mixture with a moderate amount of egg; and *C* showed the effect of adding a large amount of egg; while *D* gave a mixture with a high egg level, but of a composition similar to *B*. Formula *F* provided a dry-mixture approximating *B*, and *E* was a similar dry-mix with skim-milk protein replaced by egg protein. The effect of sugar on the keeping properties of Formulas *A*, *B*, and *C* was evaluated by drying these mixes after the addition of 3% of lactose or 3% of sucrose. While these were only a few of the possible combinations they were representative of the products likely to be used.

All mixtures were packed in hermetically sealed tin plate containers with air in the headspace and stored at 40°, 60°, 80°, 100°, 120°, and 140° F. (4°, 16°, 27°, 38°, 49°, and 60° C.). Some were packed in tin plate under carbon dioxide and stored at 80° F.

Analytical Methods

The quality of products such as these is dependent primarily on their acceptability when reconstituted as milk shakes, custards, ice cream, or other similar dishes. Therefore, the average palatability score, determined by a panel of 14 tasters, was used for assessing quality. The material was reconstituted as a milk shake mix in a manner similar to that described for milk powder (7), but using one part solids and three parts of water by weight. The products when submitted to the tasters were sweetened but unflavoured, and to prevent differences in sweetness affecting tasters' judgment, all samples were made up to a level of 7% sweetness in terms of sucrose, utilizing recently tabulated sweetness relations (2).

The scoring technique differed from that used in previous work (7, 9). A freshly prepared mix of Formula *F* was used as the standard for tasting purposes and given an arbitrary score of 10. A score of 20 indicated a product twice as acceptable as this formula, a score of 5 indicated the product half as acceptable, and a score of 0 indicated an unacceptable mix. A fresh standard sample was used as a reference whenever palatability was determined.

An attempt was made to follow solubility changes in the mixtures by a standard procedure (1). However, during storage, the decrease in solubility of all products was so slight that the test was discontinued.

The fluorescence of a saline extract of defatted egg powder has been related to the palability of this product (9), and has also shown some relation to milk powder quality (6). Later work showed only a slight correlation between this test and the palatability of milk powder (7). Nevertheless, a modification of this test (10) was applied to some of the present samples. Correlation of fluorescence values of the stored mixes with the corresponding palatability scores was lower than that obtained for egg powder alone (9). This test was discontinued since the correlation was only .4 for the most desirable sample, Formula *A*. However, the fluorescence data were of some interest and are recorded here.

The data were subjected to analyses of variance and the significant effects were selected for discussion.

Results

Palatability Changes During Storage

The palatability changes in the basic mixtures during 16 weeks' storage at temperatures from 40° to 140° F. are shown in Fig. 1. Throughout the experiment the product prepared entirely from milk solids was considered superior, both initially and after storage. At 40° and 60° F., the mixture prepared entirely from milk and the dry mix of egg powder and whole and skim-milk powder deteriorated about equally, but less rapidly than other mixtures containing egg. At the higher temperatures (80 to 140° F.) all mixtures containing egg spoiled more rapidly than the mixture prepared only from milk. At temperatures of 100° to 140° F. the material containing the greatest quantity of egg became inedible more rapidly than those containing

little or no egg. The addition of egg to milk before dehydration reduced the initial quality of the dried product and its subsequent storage life. However, the dehydrated mixture appeared to have a better storage life than has been observed for egg powder (8).

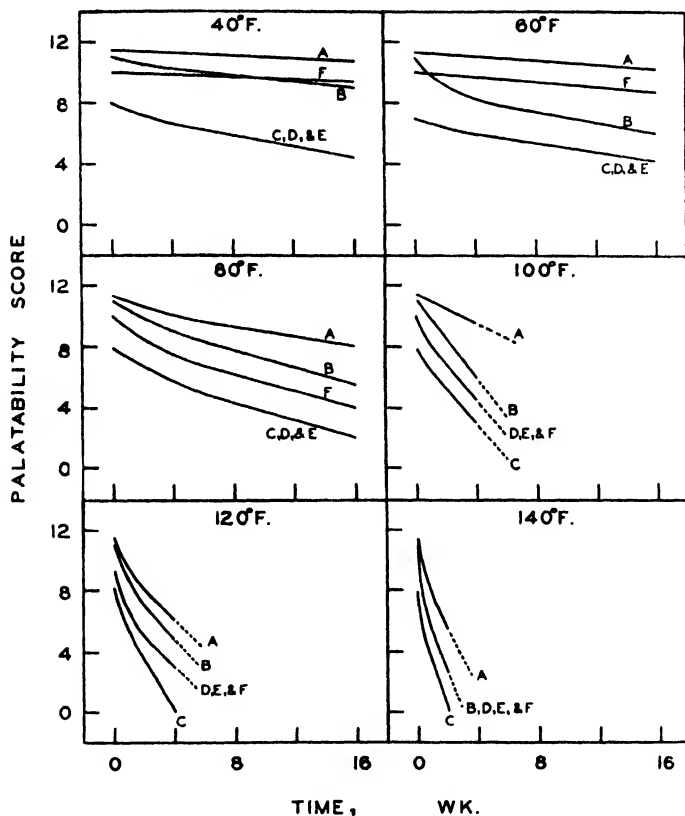


FIG. 1. Effect of storage temperature on the palatability of stored dehydrated mixtures of egg and milk.

The average values for samples with and without added sugars are shown in Fig. 2. Generally, there was a slight protective action as a result of the addition of sugar. The behaviour was not regular at all temperatures nor for all mixes (notably Mixture *B* at the higher temperatures), nevertheless, added sucrose was more effective than added lactose.

Formula *D* was similar to Formula *C* since it gave a dried product with a large quantity of egg solids, and was similar to Formula *B* in that it yielded a product with almost the same fat, protein, and carbohydrate ratio. This mixture had slightly better keeping quality at the higher temperatures than Formula *C* (Fig. 1). It was believed that this improvement was the result of adding lactose rather than the result of balancing the component ratio.

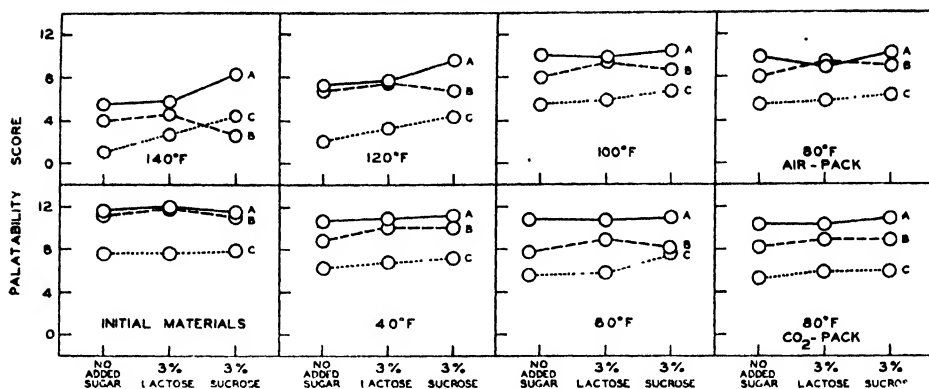


FIG. 2. Average effect of added lactose and sucrose on the palatability of stored dehydrated mixtures of egg and milk.

The comparative effects of packing some of the mixtures under atmospheres of air and carbon dioxide are shown in Fig. 3. It was observed that carbon dioxide provided some protection to the products A, B, and D, i.e., protection was afforded to mixtures of about the same composition even if eggs were

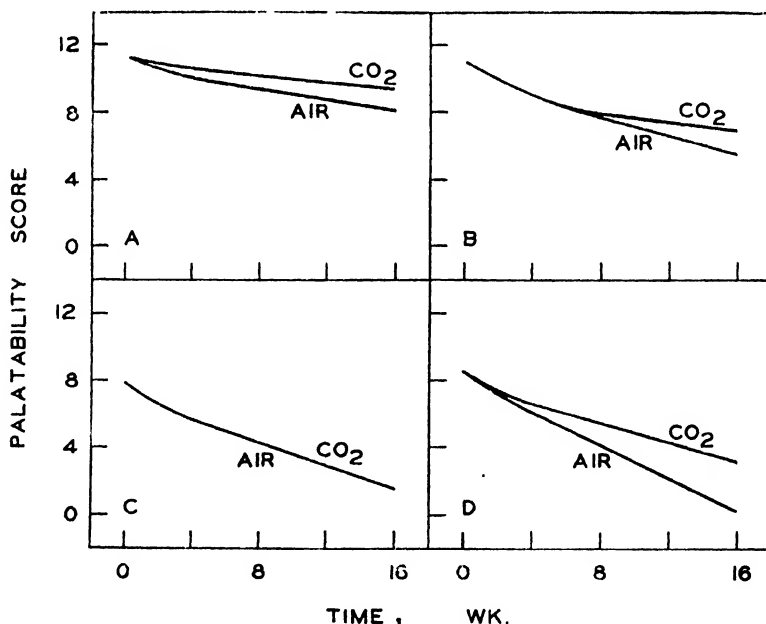


FIG. 3. Effect on palatability of packing dehydrated mixtures of egg and milk under an atmosphere of carbon dioxide; storage at 80° F.

present, but the life of material with high fat and protein from egg sources was not extended by carbon dioxide. The beneficial action of carbon dioxide was not pronounced; after 16 weeks' storage at 80° F. the palatability of

three of the gas-packed materials was about two units higher than that of the air-packed material.

Fluorescence Changes During Storage

As previously stated, fluorescence changes were measured during the initial portion of the experiment. In general, fluorescence increased with storage time and with temperature (Fig. 4). The initial fluorescence value was lower

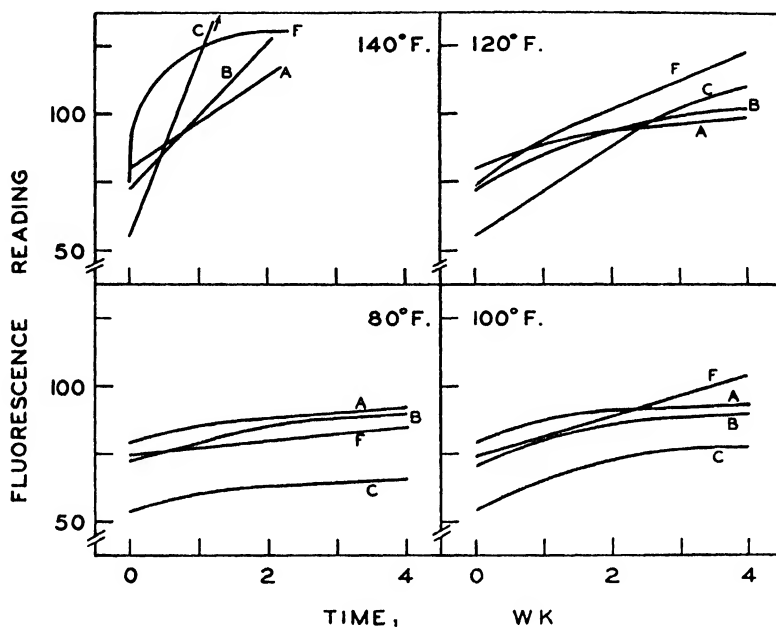


FIG. 4. Effect of storage temperature on the fluorescence reading of saline extract of stored dehydrated mixtures of egg and milk.

for mixes of higher egg content but the fluorescence value of these samples increased rapidly. Although parallel behaviour was usually noted for fluorescence increases and palatability decreases, the correlation between the two measures was less than that for egg powder alone (9).

One point of interest arises from comparison of Formulas B and F. At 100° to 140° F. the fluorescence of the dry-mix increased much more rapidly than that of the product prepared from the wet-mix, although the palatability measurements indicated about equal deterioration in these two materials.

Sucrose addition caused slight reduction in the average fluorescence value of the samples stored at temperatures of 80°, 100°, 120°, and 140° F. (Fig. 5). The greatest effect was apparent at 140° F.; sucrose caused the most pronounced reduction in fluorescence in the sample containing the greatest quantity of egg. This corresponds fairly well with similar observation from palatability scores (Fig. 2). The fluorescence measurement also indicated that sucrose was more effective than lactose.

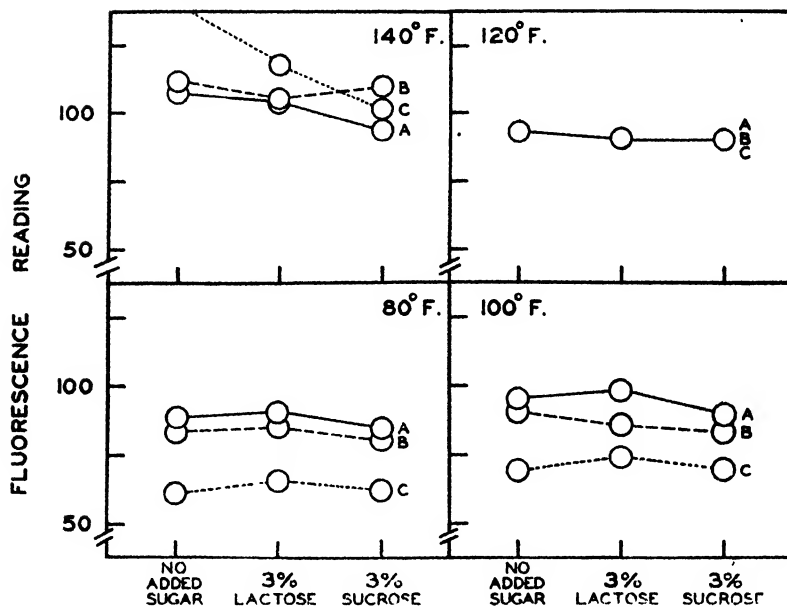


FIG. 5. Average effect of added lactose and sucrose on the fluorescence reading of saline extracts of stored dehydrated mixtures of egg and milk.

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THE OCCURRENCE AND DISTRIBUTION OF *SALMONELLA* TYPES IN FOWL

I. ISOLATION FROM HENS' EGGS¹

F. E. CHASE² AND M. L. WRIGHT³

Abstract

Using selective media, an examination of 2400 hens' eggs was made to determine the incidence of *Salmonella* types in the egg or on the shell. The contents of 1000 eggs laid by a flock of presumably normal hens did not yield any representatives of the *Salmonella* group. Of a similar number of eggs produced by a flock of 55 known pullorum reactors, 61 were found to contain *S. pullorum*; no other *Salmonella* organisms were isolated. The exteriors of an additional 400 eggs obtained from the latter source failed to produce any *Salmonella* types.

Introduction

War-time conditions have brought about a tremendous expansion in the production and use of dried whole egg powder. The reported occurrence of a number of *Salmonella* types in the commercial product (5), although apparently not constituting a dangerous hazard to health (6), has served to direct interest towards the discovery of the source of these contaminants, since their presence in food products is certainly most undesirable. Gibbons and Moore (5) have presented circumstantial evidence to indicate that hens' eggs may be the source, not only of *S. pullorum*, but of other *Salmonella* types occurring in egg powder.

There is considerable evidence to implicate other types of fowl. Tanner (15) reviewed briefly a number of reports of *Salmonella* food poisoning in which ducks' eggs were thought responsible, and Scott (14) found three flocks of ducks in which one or more of the birds laid eggs containing *Bacillus aertryke*. Edwards (3) cited the report of a food poisoning outbreak affecting 20 persons in which pigeons' eggs infected with *S. typhi-murium* var. *copenhagen* were involved. Hinshaw (9) reported the presence of *S. typhi-murium* in the ovaries, oviduct, and eggs of turkeys. For the most part, however, hens' eggs have escaped suspicion, and no reference has been found that describes the isolation of any *Salmonella* type other than *S. pullorum* from hens' ovaries or the contents of their eggs.

Because of the number of types of *Salmonella* reported in dried whole egg powder, and in the visceral organs and fecal matter of chickens, and occasionally in hens suffering from enteric infections (3, 10, 13), it was decided to determine to what extent these types were present either within the egg or in fecal contamination on the shell. This paper deals with an examination of 2400 hens' eggs for the presence of *Salmonella* types.

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Contribution from the Department of Bacteriology, Ontario Agricultural College, Guelph, Ont., with financial assistance from the National Research Council of Canada.

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Methods

Because a number of mixed *Salmonella* infections in fowls have been reported (2) it was decided to include in this study 1000 eggs produced by a flock of 55 known pullorum reactors. Another 1000 eggs were obtained from a source where every effort is made to maintain normal birds. For the sake of brevity these eggs will be referred to as "diseased" and "normal," respectively, to indicate their source. The required number of fresh eggs were obtained at weekly intervals and held at room temperature until cultured.

The procedure used in culturing the eggs was based on recommendations made by Gibbons*. The eggs were soaked for approximately five minutes in a "Diversol" solution, containing 500 p.p.m. of available chlorine, after which they were rinsed in alcohol and placed on a sterile metal rack until dry. The ends were painted with double strength tincture of iodine and the eggs were again allowed to dry. Using aseptic precautions an egg was picked up, each end opened with a flamed awl, and then placed on the mouth of a 100 ml. sterile Erlenmeyer flask containing broken glass. Compressed air filtered through sterile glass wool was used to blow the contents into the flask. After thorough mixing, the egg meat was transferred to the culture medium.

As this investigation was concerned only with members of the *Salmonella* group, selective media were utilized when culturing the eggs in order to reduce possible interfering types. All incubation was carried out at 37° C. Bacto-Tetrathionate Broth was used as the enrichment medium from which, after 24 hours' incubation, streak inoculations were made on Bacto-SS Agar plates. These were incubated and examined at 24 and again at 48 hr. for the presence of colourless colonies, which were fished to Bacto-Kligler Iron Agar slants. This medium made it possible to discard an occasional lactose fermenter that had not been clearly differentiated by the SS medium and also a type that failed to produce acid or gas from dextrose. Another complicating organism, which appeared occasionally, proved to be a slow lactose fermenter. It invariably fermented sucrose and was easily eliminated in this way. Organisms were classified in the *Salmonella* group when they satisfied the criteria outlined by Gibbons and Moore (5).

The exteriors of an additional 400 diseased eggs were examined for *Salmonella* types. Using aseptic precautions the eggs were washed with Bacto-Tetrathionate Broth, which was incubated and streaked on Bacto-SS Agar plates according to the method already described.

Because of the divergence of opinions expressed regarding any possible relation between bacterial content of eggs and season of year (8, 12), it was decided to ignore this factor and examine the diseased and normal series of eggs consecutively rather than concurrently, thereby obviating all possibility of doubt as to the origin of the cultures. The contents of the diseased eggs were cultured during July, August, and September; the normal eggs, during

* Personal communication, 1944.

October, November, and December; while the exteriors of the diseased eggs were examined at intervals during January, February, March, and April.

Results and Discussion

The bacteriological examination of 1000 eggs laid by a flock of pullorum reactors resulted in the isolation of *Salmonella* organisms from 61 of these eggs. Since these organisms were non-motile, failed to ferment maltose or dulcitol, and gave typical serological reactions, they were classified as *S. pullorum*. Of interest was the fact that 35 of these cultures, though apparently anaerogenic in dextrose broth, did show slight gas production in the butt of Bacto-Kligler Iron slants. The exteriors of 400 undamaged eggs from the reactor group were also cultured for the presence of *Salmonella* types. The media employed, although similar to those used in the examination of egg contents, failed to demonstrate the presence of organisms of the *Salmonella* group. The contents of 1000 eggs from a negative flock were also examined and were found to be free of *Salmonella* types according to the methods employed.

The bacteriological examination of 2400 hens' eggs resulted in failure to isolate any *Salmonella* types other than *S. pullorum*. The latter organism, perhaps the most difficult of all *Salmonella* types to isolate (3), was found in the contents of 6.1% of the diseased eggs, an incidence similar to that reported by a number of other workers (1, 4, 7, 11, 16). This is considered an indication that the methods used were sound and would have been suitable for the isolation of other *Salmonella* types had they been present. Watch was maintained for the possible occurrence of *Shigella gallinarum*, but it did not appear during the course of this survey.

It would appear from these results, which seem in accord with the literature on the subject, that hens' eggs are not likely to be infected with *Salmonella* types other than *S. pullorum*, though it must be admitted that failure to isolate other *Salmonella* organisms in a survey as limited as this does not necessarily indicate that such infections cannot occur. It is suggested that the evidence now obtained against fowl so closely akin to the domestic hen warrants continued study of the problem, with attention being directed towards the manner in which some of these other *Salmonella* types affect the laying hen.

Acknowledgment

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THE CROSS-VENTILATED TUNNEL SMOKEHOUSE¹

BY E. P. LINTON² AND A. L. WOOD³

Abstract

A tunnel smokehouse with an output of 3000 lb. of smoked fillets per nine hour day has been in operation about one year. The temperature, relative humidity, and smoke velocity are controlled at optimum values independent of climatic conditions. Hence the colour and shrinkage of the product may be standardized and losses from cooking and dropping of the fish avoided. The relatively short time of smoking of two to three hours reduces spoilage of the fish to a minimum with resulting improvement in quality. Power and steam consumption have been kept as low as feasible.

During the past two years experiments have been conducted into the controlled smoking of fish, and as a result of these investigations an apparatus has been put into successful operation. This equipment may be of interest not only to the fisheries engineer but to technologists concerned with the smoking or drying of food products in general.

The primary purpose of the smoke curing process as carried out along the Canadian Atlantic seaboard is to impart to the fish a pleasant salt and smoke flavour and a golden yellow colour. The greater part of the smoked fish production consists of mild cured cod fillets, finnan haddie, and kippered herring, which are sold in the Canadian and United States markets. These mild cured products are perishable and must be handled in the same manner as fresh fillets. Hard cured herring which do not require refrigeration are also prepared in quantity for the West Indian trade. The smokehouse described here has been developed primarily for preparation of the mild cured products.

The procedure for the smoke curing of fish has not changed greatly in the last 50 years (2, 3, 8). The process is carried out in three stages—brining, drying, and smoking. The problems connected with the brining and drying of fresh fish previous to smoking have been discussed by Cooper and Linton (1) and the data on the physical conditions suitable for smoking, which have been collected at this Station over a period of years, mainly by Cooper and Linton, have been published only in the reports to the trade. A review of fish smoking has been published by LeGall (5). Lemon (6) has described an experimental smokehouse but no data are given on smoking conditions. Cutting (4) has discussed the engineering problems in the smoke curing of fish in England. He has developed a kiln primarily for the preparation of English kippers which, because smoking is used as a means of preservation,

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are both dried and smoked to a greater extent than the kippered herring sold on the Canadian market.

High temperatures during warm weather (resulting in soft partially cooked fish), high humidity, unevenness of smoking, dust particles on the fish, and excessively high labour costs are some of the faults in the smoking process as now carried on. Suitable smoking conditions may be summarized as follows: temperature of fish must not rise above 75° F., the relative humidity (usually about 65 to 70% at 75° F.) regulated to give required weight loss during the smoking period, velocity of smoke about 400 ft. per min., depending on the required length of the smoking period. Smoke density or smoke concentration will be discussed in a later article.

The Design of the Smokehouse

Any one of the numerous types of tunnel or compartment dryers might be adapted for smoking fish. The most important factor to be taken into consideration is that the air and smoke flow must be uniform, that is, they must have the same velocity throughout the cross-section of the smoking space in order to produce the same colour on all the fish. At the same time it is desirable to keep the power consumed by the fans to a minimum, as in many cases these smokehouses must be installed in outlying districts where power rates are high and the power available may be limited. Taking these factors into consideration, it appeared probable that the cross-ventilated tunnel type should give reasonably uniform smoke flow with a minimum consumption of power. Because of the difficulty of predicting air flow behaviour, it was necessary to construct a full scale commercial model. The tunnel smokehouse shown in Fig. 1 is constructed mainly of wood, is double walled, and finished with $\frac{3}{4}$ in. sheathing for use where insulated installations are required. Plywood, sheet metal, or wallboard are equally satisfactory. The size of the house is determined largely by the weight of fish that can be conveniently handled on the cars. The house holds two cars each carrying about 400 lb. of cod fillets. As the smoking and drying power of the smoke stream fall off noticeably in six to eight feet of travel over the fish, this distance determines to some extent the length of the cars. For instance, four cars cannot be used in the same equipment without an intermediate addition of heat and smoke. For convenience of the operator the smokehouse is maintained at slightly less than atmospheric pressure by the exhaust fan and hence it must be well constructed, all the joints must be sealed, and doors must be reasonably smoke tight.

Mechanical Equipment

The recirculating fan used in the present smokehouse is a reversible 42-in. propeller type fan. The fan is supported on a steel shaft that protrudes through one end of the smokehouse so that the driving motor is not operated within the smoke. When construction was started it was not known which was the most desirable direction in which to have the air and smoke flow

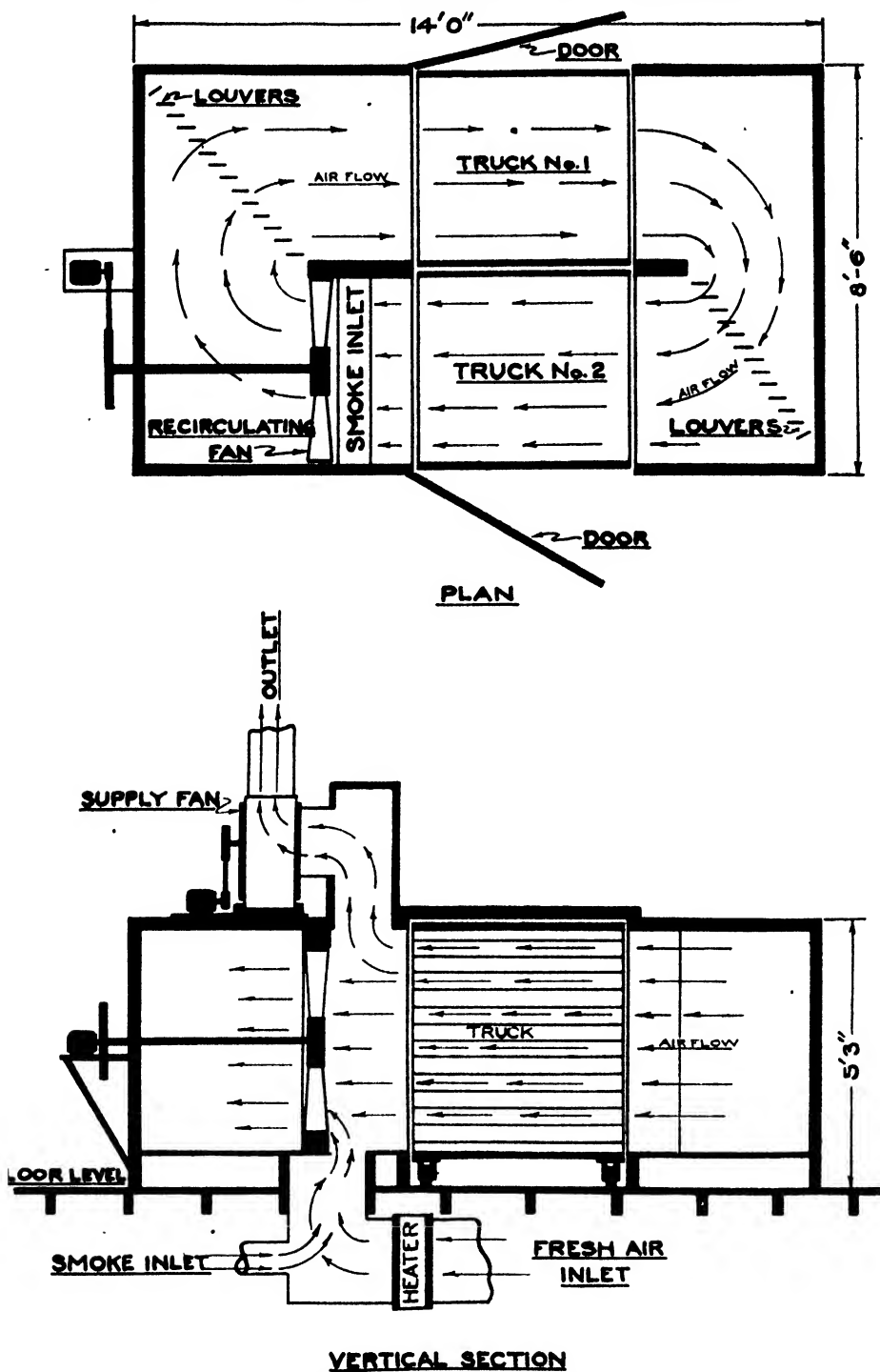


FIG. 1. The cross-ventilated tunnel smokehouse.

within the house, and, hence, the reversible type fan wheel was installed. The diameter of the fan was shown to correspond roughly with the height and width of the cars holding the fish. In the preliminary experiments the fan was driven by a 2 hp. motor using a speed reducer to give fan speeds varying from 135 to 470 r.p.m. After the preliminary experiments a $\frac{1}{2}$ hp. motor was connected to the fan with a V-belt. About 6000 cu. ft. of air per min. was recirculated by the fan.

The exhaust fan is a Clarage No. 1 $\frac{1}{2}$ driven at 990 r.p.m. by a $\frac{3}{4}$ hp. motor. A manually operated damper installed on the inlet side of the exhaust fan controls the volume of air vented to the atmosphere.

The steam heater installed on the inlet duct heats the incoming air sufficiently to maintain the smokehouse at the desired dry-bulb temperature. The dry-bulb thermostat installed in one end of the smokehouse controls the steam supplied to the heater and, hence, the temperature to which the incoming air is heated. Temperature control is maintained within 1° or 2° F. Both the dry-bulb temperature and wet-bulb temperature are recorded automatically with Brown psychrometers.

Smoke Producers

The type of smoke produced and the flavour of the fish are determined by the kind of sawdust burned, the water content of the sawdust, and the ratio of sawdust to air. For the most part, sawdust was purchased from local fish companies. It was free from strong odours such as those given off by fir or pine. The optimum water content of the sawdust was about 40%. The particle size varied from 0.1 to 0.2 in. in diameter.

Two types of burner were used. The first, a metal cone approximately six feet in height and five feet in diameter, placed over the ordinary planer-shavings sawdust fire, permitted a large volume of air to be drawn over the sawdust, giving a smoke very similar in odour and flavour to that used by the trade. This type of smoker is used commercially on installations of smoking equipment now in operation. The main disadvantage of the cone smoker is due to the inability to cool or dehumidify the air entering the smoker during warm weather. A fairly steady continuous smoke production is maintained by keeping two in constant operation, charging and relighting them alternately.

The second smoke producing equipment utilized was the Buffalo Smoke-master (John E. Smith's Sons Co., Buffalo, N.Y.). It has been used for the smoking of meats. It was found to require considerable attention and in general is not, in our opinion, as satisfactory as the cone smoker.

Smoke Density Meter

A simple type of smoke density meter was constructed with the equipment readily available. As a source of light, a 150 watt, 110 v. Mazda Projector Spot Lamp was installed in one end of the smokehouse. The path of light passed through about 40 inches of the smoke stream and then through a glass window to a Weston Photronic Foot-Candle Meter. Unaccountable variations in the intensity of the light from the Spot lamp were noted even though

a constant voltage transformer was installed in the circuit. Finally, fluctuations in the light sources were compensated for by standardizing the Weston cell meter with a 60 watt Mazda lamp installed outside the smokehouse near the Weston cell. The Weston cell was mounted on a pivoted base at a distance from the two light sources so that when swung from one to the other the cell gave the same zero reading of 200 fc. A variable voltage transformer connected in parallel with the two lamps enabled the zero reading to be set at any desired value. In measuring the smoke density, the meter was set at 200 fc. facing the 60 watt bulb and then swung to give the reading with smoke present. Most readings with smoke fell between 25 and 150 fc. but occasionally readings as low as 1 or 2 fc. were obtained. Optical density was calculated in the usual way by subtracting the logarithm of the reading with smoke present from the logarithm of the reading with no smoke in the house. The smoke density readings recorded are average values.

Temperature and Relative Humidity Control

The point at which smoke and air were admitted to the house is important, and several trials were made before the inlet and outlet were satisfactorily located. It was necessary to have the incoming fresh smoke and air mix with the recirculated smoke by passing through the recirculating fan. In this way uniform smoke and temperature distribution could be obtained across the section of the tunnel. Other points of inlet for the smoke were not satisfactory. Exhausting the smoke at a place shown in the diagram ensures that its temperature will be at a minimum and the relative humidity at a maximum, giving good economy of operation.

During the warm summer months when the dew-point exceeds 55° F. it is necessary to cool and dehumidify the fresh air entering the smokehouse along with the smoke. The smoke entering the house must not be cooled directly with ice or cooling coils as tars and smoke flavours, essential to the smoking process, are removed by this treatment. The only way to control the temperature (dry-bulb) and the relative humidity of the house is to admit a sufficient quantity of dehumidified and cooled air along with the smoke. The cooling and dehumidification may be carried out by mechanical refrigeration or by ice. In the arrangement used, the fresh air entering the house is drawn through an ice box containing 600 to 700 lb. of ice, crushed to give lumps 6 to 10 in. in diameter. This air is at a temperature of about 40° F. and is saturated with water vapour. It must then be heated by the steam heater to give the desired temperature within the smokehouse. Under the most adverse weather conditions likely to be met with in the Maritimes, about 1200 lb. of ice and 200 lb. of steam per hour will be consumed with the present equipment. The relative humidity of the smokehouse depends largely upon the volume of conditioned air mixed with the smoke entering the house. When the house is filled with fish, about 1500 cu. ft. of air per min. are mixed with about 300 cu. ft. of smoke per min. from the cone smoker.

Fish Supports

In most commercial houses the fish are supported on rods or hooks. The hanging of the fish is time consuming, and fish are often spoiled by dropping to the floor of the house. These difficulties are overcome by supporting the fish on wire-mesh trays.

The tray of most convenient size for the majority of fish products is about $2\frac{1}{2}$ by 4 ft. and consists of a metal screen supported by a welded steel frame. When trays are used to support the fish, the marks of the wire are quite noticeable on the fish. Fillets are placed with the skin side down on the smoking trays, while herring are placed with the cut side next to the screen. Fish are sometimes dyed with harmless dyes, to increase the colour. On such fish the marks of the trays are scarcely noticeable. The loading capacity of the tunnel depends primarily on the spacing of the trays on the cars and on the size of the fish being smoked. For cod fillets the trays should be placed one above another about $3\frac{1}{2}$ in. apart. Large steak cod will give a loading of about 4 lb. per sq. ft. of tray area. With market cod, loading is about 2 to $2\frac{1}{2}$ lb. per sq. ft. and with scrod, about $1\frac{1}{2}$ lb. Fillets are loaded on the trays with their length in the same direction as the air flow. With kippers the trays may be placed closer together on the cars and the trays are loaded to about $1\frac{1}{4}$ lb. per square foot. Finnan haddie give about the same loading per square foot as cod fillets. Although the fish may be hung on rods that are supported on the cars in somewhat the same manner as in the present commercial houses, the loading capacity of the tunnel smokehouse will be reduced by about one-third. Furthermore, considerably more labour is required for hanging the fish on rods than simply placing them on the trays.

When the trays are used day after day for the smoking of fish, a considerable deposit of smoke tars and particles of fish accumulate on them. Furthermore, they tend to rust and may leave undesirable rust marks on the fish. These difficulties are best avoided by waxing them periodically. When the trays are new they are dipped in hot paraffin wax and allowed to drain. Other waxes, such as Opalwax, manufactured by Canadian Industries Limited, which melts at 176° F. may be used, or an aqueous suspension of Johnson's Clear Rust Inhibiting Wax No. 1568 may be applied directly to the trays. The ordinary paraffin wax appears to be the most economical of those tried.

Air Flow and Power Consumption

Studies were made on the air flow to determine the best direction of flow and the most suitable means of ensuring even distribution across the cars.

When the air and smoke were blown directly onto the first car of fish a dead air space in front of the hub of the recirculating fan was formed. Also the rotating blades collected tar and soot, later throwing them onto the fish. Therefore the rotation of the fan was reversed. This changed flow permitted the thorough mixing of the incoming smoke with that being recirculated.

Three different types of devices for straightening the air flow were investigated. The results of air flow measurements and wattage consumed for each device are shown in Table I. Semicircular splitters were installed in the first trials. As the table shows, they are quite efficient and possibly require slightly less horsepower than the other devices used. However, as they are

TABLE I

WATTS REQUIRED BY DIFFERENT TYPES OF AIR FLOW STRAIGHTENERS

Fan, r.p.m.	Semicircular splitters		Wooden louvers		Grids	
	Air velocity, ft./min.	Watts	Air velocity, ft./min.	Watts	Air velocity, ft./min.	Watts
135	325	120	350	30	200	70
260	650	270	600	170	375	270
326	900	430	770	350	450	470
400	1100	760	1000	680	600	860
470	1300	1200	1100	1120	725	1330

rather difficult to install and construct, they were abandoned in favour of the wooden louvers. The wooden louvers were 4 in. wide and $\frac{1}{2}$ in. thick and were set on pivots four inches apart so that they could be adjusted to direct the air flow where required. This device is simple to construct and gives uniformity of air flow. A third trial was made with an arrangement of grids constructed of wallboard. The grids were about 3 in. wide and were more or less uniformly spaced across the face of the tunnel, the spacing arranged to give uniformity of air flow. It was found practically impossible to avoid dead air spaces, and furthermore, as the table shows, the wattage consumed for a given air velocity was considerably greater than with either the semicircular splitters or the wooden louvers. With wooden louvers installed as shown at both ends of the smokehouse, the air velocity was found to range from 400 to 500 ft. per min. across the section of the house where the fish are placed. The variation of 100 ft. per min. is not considered to be significant in view of the fact that the cars pass periodically through the house. At this air velocity $\frac{1}{2}$ hp. was consumed by the recirculating fan.

In an effort to keep the house to a minimum size, experiments were made to shorten it by erecting false walls at various positions in the two ends of the house. It was found necessary to have the wall at the inlet end about $3\frac{1}{2}$ ft. from the fan, and at the other end about 40 in. from the cars of fish in order to obtain satisfactory air flow.

The exhaust fan is capable of handling about 2000 cu. ft. of air per min. against a one inch static pressure. About 390 watts is required by the motor operating this fan.

The Experimental Smoking of Fish

Smoking experiments carried out in the tunnel smokehouse, using various types of fish and smoking conditions, have demonstrated the uniformity of smoke flow in the house, and have established the effect of some of the factors concerning the smoking, such as pre-drying and smoke velocity. The results are given in part below and in part by Linton and French (7).

The uniformity of air flow has considerable influence on the evenness of drying and smoking. In a typical experiment 800 lb. of cod fillets, obtained from a local fish company, were smoked for two and one-half hours, the dry-bulb temperature being 76° F., and the relative humidity, 70%. The cone smoker was used as the source of smoke and about 1000 to 1500 cu. ft. of air and smoke were exhausted from the tunnel per minute. Trays of the fish at various points in the trucks were weighed before and after smoking. The average weight loss of the cod fillets was 11.4% and the extreme range of weight loss in the weighed trays of fish was from 12.2 to 10.5%. In view of the fact that the fillets were not of the same size, this variation is not large enough to be of any importance. Furthermore, on examination the next morning, the variations in the colour of the fillets were scarcely noticeable. The fish were packed for sale by a local company and all of them were packed out as first quality. In commercial practice, fish are sorted into three sizes before smoking, as fish of different sizes smoke and dry to a different degree under the same smoking conditions. With this sizing, differences in colour would not be detectable.

In the operation of the present commercial smokehouse it is customary to allow the fish to drain an hour or so, and then dry for one or two hours over open wood fires until the surface is partially dried and the fish are sticky to the touch. The weight loss with cod fillets is usually 5 to 7% during this pre-drying. Pre-drying is considered to be an essential part of the smoking procedure in order that a glossy surface may be formed on the fish. The pre-drying may be carried out in the present tunnel smokehouse in 40 to 60 min., but it is also possible owing to the higher smoke velocity used in the tunnel smokehouse to omit the pre-drying step for fillets and smoke them after they have drained for a few minutes. An attempt was made to determine whether there was any appreciable difference between the appearance of fillets that had been pre-dried and those that had been smoked without preliminary drying. Eight hundred pounds of market size cod fillets were loaded on the two cars and the fish in one car were dried for 50 min., resulting in a weight loss of 4 to 5%. Both cars were then smoked for three hours at a dry-bulb temperature of 75° F. and about 73% relative humidity. On packing out the fish from the cars there was very little difference, if any, in the surface appearance of those processed by the two different methods. Obviously there was a slightly higher weight loss of 1 to 2% in the fillets that had been pre-dried. From this experiment it was concluded that with a thin smoke from the cone smoker (average density, 0.20), it is not necessary to pre-dry fillets previous to smoking. In some cases, where a relatively

large weight loss is desired it may be necessary to pre-dry the fish. The pre-dried fish do not have as deep a colour for the same time of smoking as the undried fish.

In the majority of the fish smoking experiments the velocity was set at about 450 ft. per min. Using this smoke velocity, the power was maintained at a value that could be drawn from local lighting circuits, and furthermore, it gave a considerable decrease in the smoking time over the present commercial methods. As indicated above, sufficient colour was obtained in the cod fillets in two to three hours at 450 ft. per min. smoke velocity. In one experiment smoke velocity was decreased to 125 ft. per min. and this lengthened the smoking period to five to six hours to give the same depth of colour, other conditions being the same.

Other species of fish—herring, haddock, and mackerel—have been smoked in the house with very good results. In commercial practice these fish are hung from hooks and this may be done in the present type of smokehouse. However, in our experiment they were laid flat on the metal trays. Herring are placed cut side down on the metal surface, so that when packed, the tray marks do not show on the skin surface. Kippered herring acquire sufficient colour in about three hours and lose about 15% in weight at a relative humidity of 65% and a smoke velocity of 450 ft. per min. in the smoking period.

Several experiments were carried out on the smoking of herring fillets for canning purposes. The fillets are laid skin side down on the trays and acquire sufficient smoke in 40 to 60 min. to give the desirable golden yellow colour on the skin of the fillet. They may be dried to any extent by controlling the relative humidity within the house, by the admission of conditioned air or, if necessary, by pre-drying.

In continuous operation the cars of fish are moved through the house at time intervals equal to one-half of the required smoking period. As each car remains in each position in the smokehouse for one-half of the smoking time, uneven air flow in one position is largely compensated for in the second position. Furthermore, the direction of smoke flow with respect to fish is reversed on moving the cars from one position to the other and all sides of the fish are smoked to the same extent. Increased capacity is obtained by installing several units side by side so that each car runs through one, two, or more units as required.

This method of smoking fish is essentially continuous as contrasted with the batch operation of the present commercial houses. This is a considerable advantage in lowering labour costs. Furthermore the fish do not deteriorate in quality to such an extent during the greatly shortened time of smoking.

The tunnel smokehouse has been in operation for about a year, and no serious defects have become apparent. One objection, the importance of which cannot be forecast definitely, lies in the fact that the development of colour is rather a slow reaction, maximum colour being reached after one to three days, depending on the temperature. Hence it is somewhat difficult for the operator to determine when the fish are sufficiently smoked.

On the other hand, it approximates continuous operation, cars being removed in little less than hourly intervals. Because of the very materially increased smoking rate and of the greater capacity of the effective smoking space, the output per cubic foot per hour is of the order of 50 times that of the regular smokehouse. Its construction is inexpensive and its operation is simple. The equipment should be of definite assistance to the producers and processors of smoked fish.

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A COMPACT CARBON PILE RHEOSTAT¹

J. T. BURT-GERRANS²

Abstract

This paper describes a carbon compression rheostat having, in addition to all the advantages set out in the handbooks of electricity and engineering, compactness and great steadiness of operation. The method of operation is detailed and a graph of a performance test is included.

Introduction

General discussions of carbon compression rheostats with citations of advantages and fields of application are published in handbooks of electricity and engineering, e.g., Marks (1, p. 1593), Pender (2, pp. 1233-1234), and Pender and Del Mar (3, 4-08), but, in the literature, descriptions of particular forms are few. The rheostat herein described was developed in Toronto for use in electric furnace work about twenty-five or more years ago and has been used successfully, as occasion arose, ever since. Rheostats based on this pattern have been in use also in the laboratories of the National Research Council, Ottawa, for upwards of 10 years.

The apparatus is particularly adapted to low voltage electrolytic work, because of the very fine stepless adjustments obtainable. With it, currents up to 1200 amp. may be continuously varied and controlled by the turning of a threaded brass or copper pressure rod. Although the rheostat works best on low voltages, it may be used on any voltage up to 110 a.c. or on the same high d.c., provided that the cooling water is neither hard nor sufficiently loaded with purifying or softening chemicals to render it conducting. Most industrial waters, however, are suitable.

Description

In Figs. 1 and 2 it may be seen that a standard asbestos pipe 4 in. I.D. \times 24 in. long is filled with carbon plates $\frac{1}{4}$ by 3 by 3 in., cut into U-shape* and stacked as shown in plan (Fig. 1, C). This arrangement provides a central vertical channel, a slot in each side of each plate and eight periferal vertical channels for the cooling water. The whole, together with two brass pressure equalizer plates, is held between two flanged and gasketed brass castings or turned plates with lugs (Fig. 1, B), with six $\frac{1}{4}$ in. iron tie rods. The upper equalizer plate is $\frac{3}{8}$ by 3 by 3 in., and the lower $\frac{3}{8}$ by $3\frac{7}{8}$ in. in diameter, centre bored with a 1 in. hole. The tie rods are insulated from the castings by means of rubber bushings and fibre washers. The gaskets, shown only in

¹ Manuscript received July 30, 1945.

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* All plates were obtained from the Canadian National Carbon Company, Toronto, Canada, those lately supplied being reedy cut.

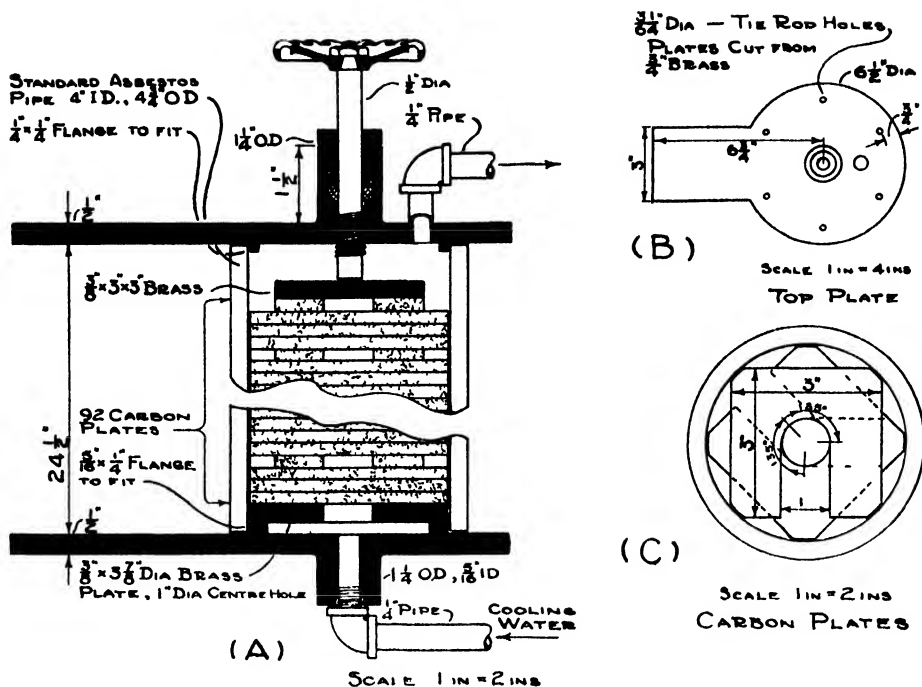


FIG 1 (A) Vertical section (B) Casting detail, plan (C) Arrangement of carbon plates, plan

Fig. 2, are of rubber, or steam packing, and seal the joints between pipe and castings. The packing gland (Fig 1, A) prevents water leakage around the pressure rod. Electrical connections are clamped or bolted to the lugs and water connections made with 1/2 in rubber garden hose.

Operation

In operation, water enters at the bottom, passes upward in the central channel through the slots into the vertical periferal channels and out through the top. The flow should be about 15 litres per minute, depending on the amount of power to be absorbed. This should be the first adjustment. With the pressure rod loosened to its limit, the rest of the circuit is completed and then the rod screwed down until it touches the plates. Thereafter, the change in pressure on the plates caused by the slightest turning of the rod is indicated by an ammeter in the circuit. Only one complete turn of the rod is required to cover the whole range of pressure. When the rheostat is not in use the pressure should always be released, otherwise the plates are apt to stick together. Though considerable pressure may be applied safely, it is possible to break the plates by screwing them down too tightly; this should be kept in mind. When the applied voltage is 50 or more, currents below 50 amp. are apt to be unsteady because of the low pressure required. Below 50 v., however, the currents are quite steady.

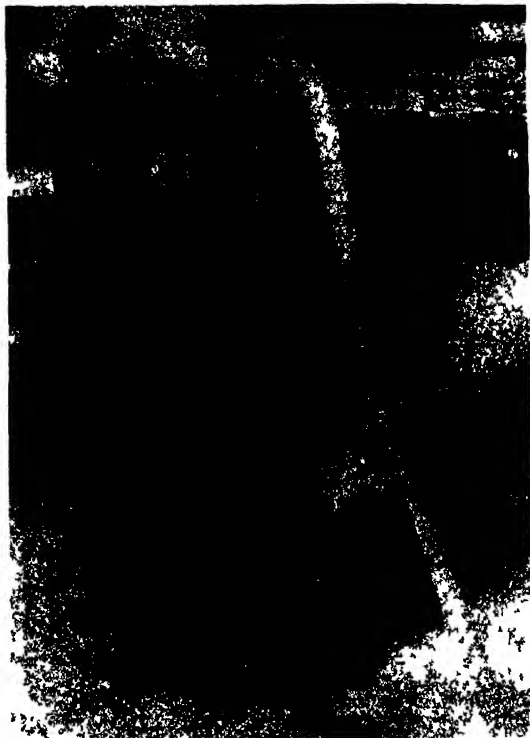


FIG 2 Rheostat assembled

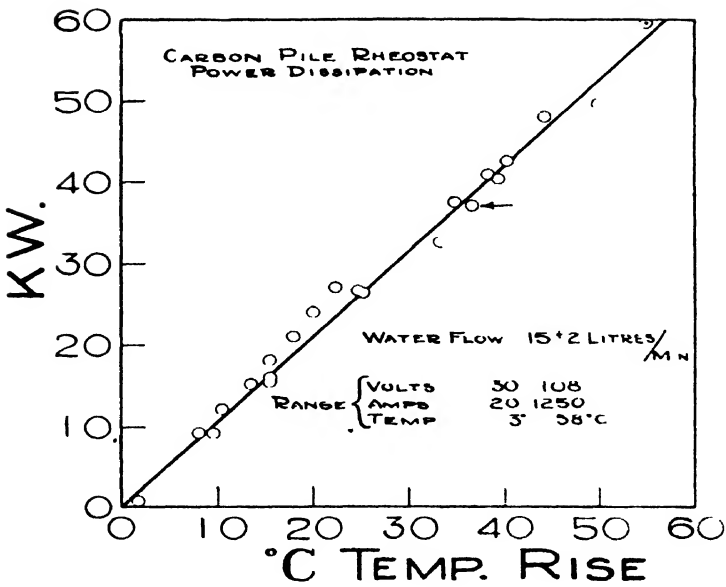


FIG 3 Graph of performance.

Performance

Fig 3 is a graph of the results of a series of measurements made when the rheostat was connected to a 25 cycle a c transformer, variable between 30 and 120 v. in 3 and 6 v steps The line represents the theoretical kw values for a flow of 15 litres per minute it has a slope of 1 kw per degree rise in temperature of the water The point marked with an arrow represents the observed value for 1250 amp at 30 v

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RESIN-RUBBER FROM CANADIAN GROWN PLANTS

IV. ANALYTICAL STUDY OF MILKWEED POD GUM¹

By R. W. WATSON² AND N. LEVITIN³

Abstract

Fresh pod gum has an acetone-soluble content (resin) of 83.4%, and a benzene-soluble content of 7.3%. Steam distillation of the resin furnishes a small amount (0.1%) of an essential oil. Acidification of the saponifiable portion (55%) of the resin yields an oil consisting of a mixture of fatty acids. Palmitic, stearic, oleic, linoleic, and linolenic acids were identified. Resin acids are absent. The unsaponifiable portion (45%) contains a large amount of α -amyrin, and extremely small amounts of sterol (probably heterogeneous) and resene. Polyisoprene, an oil containing free acids, and an unsaponifiable residue make up the benzene extract. Water-solubles include calcium tartrate, soaps, and a material yielding a mixture of sugars on hydrolysis. The residue comprises lignin, cellulose, protein, and inorganic materials. From these data the dry gum contains approximately 50% of fatty acid esters, 26% of α -amyrin, 6% of polyisoprene, 4% of ash, 3% of lignin, 2% of cellulose, and small amounts of protein, sterol, resene, essential oil, and calcium tartrate. Unidentified substances comprise 7.7%.

Introduction

Recent interest in the production of milkweed floss has led to a study of possible by-products from the waste hulls. The following paper in this series will deal with the extraction of a resin-rubber gum from alkali-digested pod hulls of the common milkweed, *Asclepias syriaca* L. (20). Little is known concerning the chemical composition of the raw material, and the gum itself has not hitherto been investigated.

Besides the fruit walls a varying proportion of seeds, floss, and placentae occurs in the material from which the gum was mechanically extracted. Rheineck (17) in 1939 reported the first chemical investigation of the follicle walls from which an ester (believed to be the propionate) of α -amyrin, and a hydrocarbon ($C_{25}H_{52}$; m.p. 63° C.) were identified. The presence of a polymerized hydrocarbon (C_5H_8)_n, assumed to be the *cis*-configuration (rubber) of polyisoprene, was also reported. It has since been shown that the polymer present in the stem-latex of milkweed is true rubber (14). Linoleic acid has been identified, and a sterol has been isolated and characterized from the oil extracted from milkweed seeds by petroleum ether (17). Rheineck has also isolated a sterol from the seed hairs. So far no chemical study of the placentae seems to have been made.

Experimental and Discussion

Preliminary acetone extractions, drying of the residues, and subsequent benzene extractions of small samples (4 gm.) of dry gum were conducted in

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa, Canada. Issued as Paper No. 33 on the Industrial Utilization of Wastes and Surpluses, and as N. R. C. No. 1364.

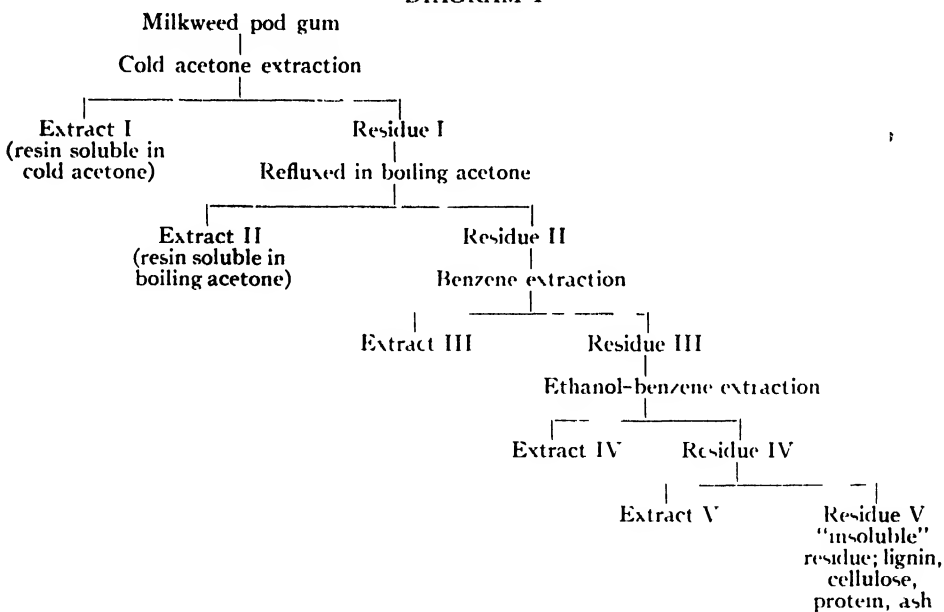
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Soxhlets, to which clean flasks with fresh solvent were attached at intervals. Hyperbolic extraction curves were obtained, becoming asymptotic for acetone at about 200 hr., and for subsequent benzene extraction, at about 100 hr. In this way at least 83.4% of the gum was found to be acetone-soluble (resin) and 7.3% benzene-soluble.

The resin for this investigation was obtained by thorough extraction with (a) cold acetone and (b) boiling acetone of about 1540 gm. of fresh gum containing 20.3% of water. To avoid prolonged heating of the extracts in the boiling flask, fresh solvent was substituted at intervals. The residue from the cold acetone extraction was refluxed for 16 hr. with boiling acetone, and then extracted successively in large Soxhlets with benzene, azeotropic ethanol-benzene, and water. The solvent fractionation of the gum is outlined, and the extracts and residues numbered in Diagram I.

DIAGRAM I



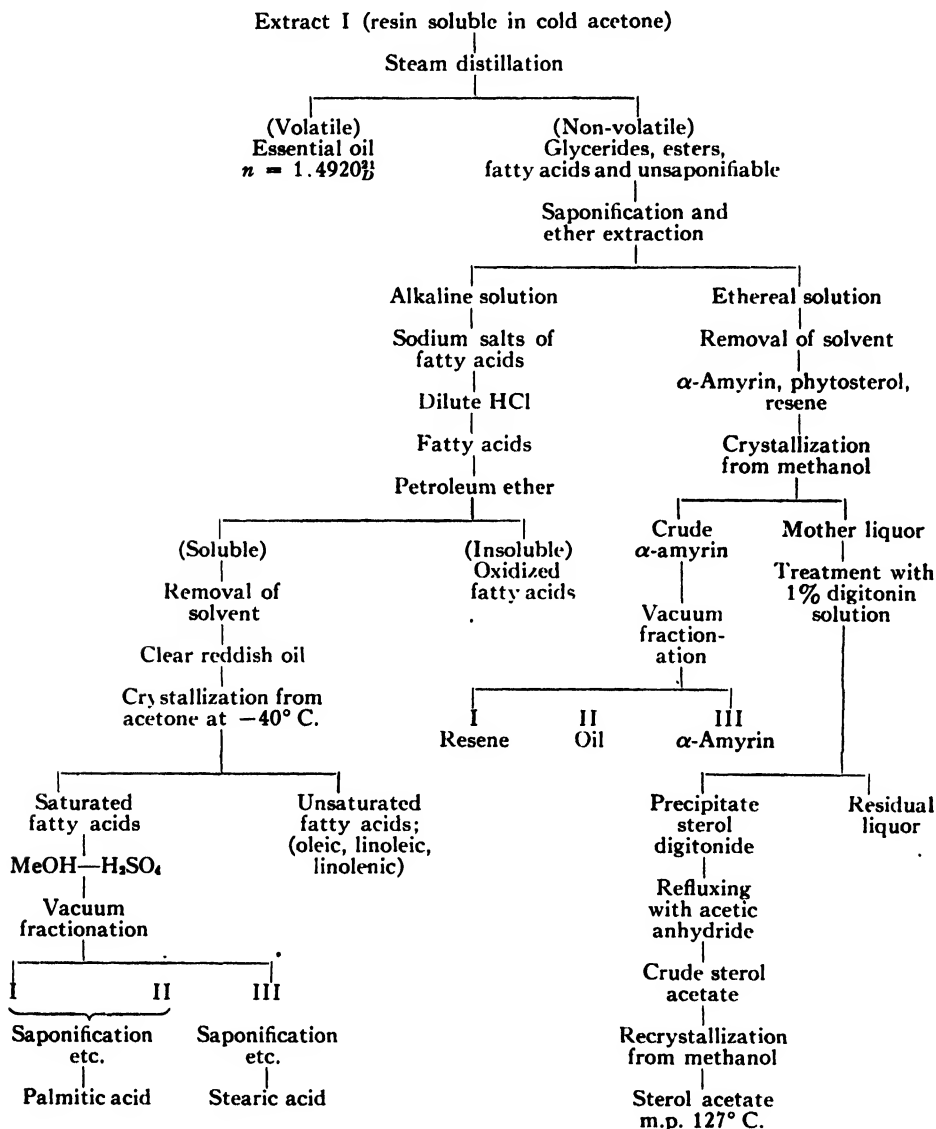
COLD ACETONE EXTRACT I (see Diagram I)

The cold acetone extracts were combined, the solvent recovered under partial vacuum (nitrogen), and the residue dissolved in ether. After washing to neutrality with water the ethereal solution was dried over sodium sulphate and Drierite. Water soluble material in the resin is negligible. Distillation of the ether yielded a soft yellow resin, with an iodine absorption value of 95.2*, an acid value of 10.5, and a saponification value of 139.3. Extraction with several portions of 5% aqueous sodium bicarbonate, combination of the extracts, acidification and re-extraction with ether yielded 0.17% of colourless semisolid fatty material.

*All iodine numbers were determined by the Wajs method.

About 160 gm. of the neutral cold acetone extract, dispersed in hot water and subjected to steam distillation for seven hours, yielded about 1500 ml. of a slightly milky distillate. After shaking the distillate with ether, and drying the ethereal solution over sodium sulphate, a small amount (0.2 gm.) of a yellowish brown oil ($n = 1.4920_D^{21}$) was obtained on recovery of the solvent. This oil possessed a strong characteristic odour, and was present to the extent of about 0.1% in the gum. The further separation of the cold acetone extract into groups of substances is outlined in Diagram II.

DIAGRAM II



Saponification of Esters

The residual resin after steam distillation consisted of a mixture of glycerides or other esters of fatty acids, α -amyrin, and sterol, along with resene and unidentified resinous substances. The saponifiable fraction (55%), a brown turbid oil, was quantitatively determined by the modified Kerr-Sorber method (7). To obtain a quantity of this oil, about 157 gm. of washed dry resin was dissolved in enough 95% ethanol to make a 25% solution, and refluxed with potassium ethanolate for one hour. After rapid cooling, the mixture was diluted with ether in a large separatory funnel. On extracting with several portions of 0.2 *N* aqueous potassium hydroxide, filtering, and acidifying with hydrochloric acid, a considerable quantity of the same turbid oil was obtained. Extraction with ether and drying in the usual way yielded 91.1 gm. (58%). A light brown solid settled out on standing.

The ethereal solution of unsaponifiable substances was washed to neutrality with water, and dried over sodium sulphate, and Drierite. Removal of the ether left a cream-coloured plaster-like solid, comprising 42% of the resin.

The fatty acid oil was clarified by taking it up in sufficient light petroleum ether to make a 10% solution, and filtering. This treatment removed 5.5% of some insoluble material, which was not further investigated. The filtrate was refluxed with activated charcoal, filtered, and shaken at intervals for several days with diatomaceous earth. This procedure failed to remove the colouring matter, and on recovery of the solvent, a clear reddish oil with the characteristic odour of the essential oil remained. The constants of this fatty acid oil are given in Table I.

TABLE I
CONSTANTS AND COMPOSITION OF MILKWEEED OILS

	Seed oil	Pod gum oil
Acid value	11.0	184.4
Hexabromide value	0.0	3.2
Iodine value	122.8	112.5
Refractive index	1.4730 _D ²⁵	1.4729 _D ²⁵
Saponification value	191.9	191.3
Specific gravity	0.9221 ₂₅ ²⁵	0.9177 ₂₅ ²⁵
Linoleic acid, %	43.8	35.4
Linolenic acid, %	0.0	2.8
Oleic acid, %	51.8	55.3
Saturated acids, %	4.4	6.5

The fruit walls of *A. syriaca*, according to Rheineck, contain a fixed oil, and several investigators (6, 10, 13) have reported the presence of about 20% of a semidrying oil in the seeds. The constants of the seed oil, as determined by Lanson, Habib, and Spoerri (10) are compared in Table I with those of the pod gum oil from this investigation. The similarity seems to indicate that the oil which forms such a large proportion of the gum may come from seeds present in the raw material.

Four separate esterifications with methanolic sulphuric acid (23) invariably failed to yield a measurable acidic residue. This observation, taken in conjunction with the amount and character of the material removed from the resin by 5% aqueous sodium bicarbonate, conclusively demonstrates the absence of resin acids. Addition to the oil of excess ether or ethanol yielded faintly turbid solutions from which a trace of a white solid separated on standing. On the addition of a small amount of 1% digitonin in 90% ethanol to a hot ethanolic solution of the oil, a few crystals of digitonin steride separated after several days. The presence of this trace of sterol is due in part to its solubility in unsaponified oil, and also to the inefficiency of extraction in large separatory funnels.

Separation and Identification of the Fatty Acids

Vacuum fractionation in a semimicro column (1) of the methyl esters of 1.3 gm. of saturated acids, separated by crystallization from the oil in acetone at -40°C . (4), yielded three fractions. Fraction 1 was a colourless oil (m.p. 17°C .)*. Fraction 2 consisted of a colourless crystalline solid (m.p. 22°C .). On combining Fractions 1 and 2, and saponifying with potassium alcoholate, the reconstituted acid, after recrystallization from acetone at -40°C ., had a melting point of 62°C ., and proved to be identical with palmitic acid. Calc. for $\text{C}_{15}\text{H}_{31}\text{COOH}$: C, 75.01; H, 12.50%. Found: C, 74.80; H, 12.42%. Fraction 3 was a light yellow, crystalline solid, which melted sharply at 39°C . After saponification, and regeneration of the acid, it was twice crystallized from acetone at -40°C ., and once from 94.4% ethanol at 0°C . This yielded a small quantity of stearic acid, which melted at 70.6°C . Several times as much palmitic as stearic acid was present.

The residual acids from the separation of the saturated acids were recovered by removal of the acetone in an atmosphere of carbon dioxide, and taken up in ether. The hexabromide value (3) was found to be 3.25, corresponding to a linolenic acid content of 1.2%. Tetrabromostearic acid, crystallized from the brominated ethereal solution, after three crystallizations from petroleum ether, had a constant melting point of 114.1°C ., thus proving the presence of linoleic acid. Dihydroxystearic acid, prepared according to the method of Hazura (5), and crystallized twice from ether, and once from ethanol, had a melting point of 116.1°C . Calc. for $\text{C}_{18}\text{H}_{36}\text{O}_4$: C, 68.36; H, 11.40%. Found: C, 68.35; H, 11.87%. The anomalous melting point was therefore due to mixture of the α - and β - forms, and the presence of oleic acid is established.

Composition of the Oil

The method of Brown and Stoner (2) as modified for soy bean oil by Earle and Milner (4) was used in the direct quantitative determination of the saturated fatty acids. Three crystallizations from acetone at -40°C . yielded 8.6% of a near-white solid, with an iodine absorption value of 12.8. Assuming

* All melting points are corrected.

the only remaining unsaturated acid to be oleic, the presence of 7.4% of saturated acids in the oil is indicated.

A preliminary attempt to determine the composition of the mixed fatty acids was made by the spectrophotometric method of Mitchell, Kraybill, and Zscheile (12). Triplicate determinations showed close agreement, and gave an average specific α of 32.7 at 2340 Å, corresponding to 37.7% of linoleic acid, and a specific α of 1.5 at the 2680 Å peak, proving the presence of linolenic acid (2.8%). Calculation from the iodine value (112.5) of the mixture, showed 40.7% of oleic acid and 18.8% of saturated acids by difference. The discrepancy in the values for the saturated acids drew attention to the possible presence of some impurity, soluble in acetone at -40°C ., and possessing low unsaturation.

A sample of the oil was further purified by conversion of the acids to the methyl esters, distillation, and reconstitution of the acids. About 11.5 gm. of the mixed acids was esterified and distilled (180°C . (0.02 mm.)) using tared flasks. A dark tarry residue, amounting to 13.9% of the original mixture, remained in the distillation flask. The regenerated oil was of a pale straw colour (iodine value 121.4), and, when analysed by the spectrophotometric method (12), gave the composition reported in Table I. The proportions of saturated acids revealed by the direct and indirect methods were now in fair agreement.

Unsaponifiables

After removal of the free fatty acids, glycerides, and much of the essential oil by saponification and steam distillation, the unsaponifiable residue contained α -amyrin, and one or more sterols and resenes, besides uncrystallizable resinous substances.

Isolation and Identification of α -Amyrin

An accurate method for determining the amyrin content of milkweed resin was developed. This procedure utilized prolonged vacuum sublimation (160°C . (0.1 mm.)) in a special apparatus to be described in a later report, and showed the presence of at least 69.53% of α -amyrin in the total unsaponifiable residue. The preparation of a considerable quantity of pure amyrin involved refluxing the unsaponifiable residue (52.2 gm.) in sufficient methanol to effect complete solution. Crystallization was complete after two weeks, and the crude amyrin was separated by filtration, washed, and dried. It was then subjected to vacuum sublimation, and the product fractionated by further sublimation *in vacuo*. The purest fraction melted at 184.3°C . Calc. for $\text{C}_{30}\text{H}_{48}\text{OH}$: C, 84.50; H, 11.74%. Found: C, 84.45; H, 11.74%. Mol. wt. 417 (16).

Fractional sublimation (160°C . (5 mm.)) of the crude amyrin obtained by a single crystallization from methanol yielded three fractions. Fraction 1 was a colourless oil, which was not further studied. Fraction 2 consisted of colourless acicular crystals, m.p. about 50°C ., which were not identified owing to paucity of material. Fraction 3 comprised about 99% of the combined fractions and consisted of α -amyrin.

Isolation of Sterol

After repeated concentration and crystallization of α -amyrin from the mother liquor, the residual liquor was diluted with 95% ethanol, and any sterol present precipitated, after long standing, as the digitonide. A small quantity (0.75 gm.) of dry digitonin steride, corresponding to 0.3% of sterol in the unsaponifiable residue, was collected. The digitonide was refluxed for one hour with acetic anhydride, the mixture poured into excess water, and the sterol acetate twice crystallized from methanol. Its melting point was then constant and sharp at 127° C. This material gave positive tests with Whitby's colour reactions *A* and *B* (22). Calc. for $C_{27}H_{46}O_2$: C, 80.60; H, 11.45%. Found: C, 80.61; H, 11.43%. Attempts to obtain anything further of a definite nature from the residual mother liquor failed.

Sterols occur in most plants as mixtures of closely related compounds with similar solubilities, as shown by the work of Lobert (11), Thornton, Kraybill, and Mitchell (19), King and Ball (9), Holden (8), and others. The present sterol in all probability is also a mixture, and the attempt to characterize it through the acetate is merely provisional. Successive hot saponifications (20) had presumably reduced any amyirin or sterol esters in the original material to the corresponding alcohol.

HOT ACETONE EXTRACT II (see Diagram I)

From the material extracted by boiling acetone a small quantity of a fixed oil and resene were separated. Removal of acetone from the combined extracts by distillation under partial vacuum (nitrogen) left a small quantity (5.9 gm.) of a clear viscous resin with a strong odour of the essential oil. This extract represented 0.5% of the original weight of dry gum.

About half this entire quantity (2.8 gm.) was taken up in ether to make a 25% solution, and a small amount of suspended matter removed by filtration. The filtrate was extracted with several portions of 5% aqueous sodium bicarbonate. Acidification, extraction with ether, and removal of the solvent, left a small amount (0.14 gm.) of yellowish brown oil ($n = 1.4917^{25}_D$) with a strong odour of the essential oil.

Isolation of Resene

Further treatment of the remaining ethereal solution resulted in the separation of the resene. In view of the unknown structure of this compound, Tschirch and Stock's term is retained as most appropriate. Recovery of the ether, saponification, and extraction with dilute aqueous potassium hydroxide gave on acidification a considerable quantity (31.5%) of a viscous dark brown oil ($n = 1.4830^{25}_D$, iodine absorption value 142.5) with the odour of the essential oil. An insoluble fraction, which collected at the interface, yielded, on recovery of the solvent, a mixture (16.9%) of colourless waxy films imbedded in a resinous matrix. The waxy substance forming these films is the resene referred to below. Nothing further of a definite nature was obtained from this fraction.

The yellow ethereal solution of unsaponifiables left a clear brown resin on recovery of the solvent. Attempts to resolve this material failed.

Vacuum distillation (180° C. (0.005 mm.)) of a sample of the hot acetone extract yielded two fractions. Fraction 1 was a yellow mobile oil. Fraction 2 consisted of a translucent resene. The latter was recrystallized several times from methanol, from which it separated as shining scales (m.p. 66° C.). Owing to lack of sufficient material it was not subjected to elementary analysis.

BENZENE EXTRACT III (see Diagram I)

The gum fraction soluble in benzene (7.3%) included free acids, a fixed oil, polymerized hydrocarbon, and a resinous unsaponifiable residue containing resene. The extracts were removed at intervals, and fresh solvent was added in order to avoid prolonged exposure to high temperatures. After thorough extraction the various portions were combined and concentrated under partial vacuum.

A sample (100 ml.) of the concentrate was treated with several times its volume of cold acetone, whereupon a light brown precipitate of the polymer separated. Collected, washed and dried, this impure hydrocarbon weighed 7.3 gm. The dry residue from the filtrate was taken up in ether, and extracted in the usual way with 5% sodium bicarbonate solution, yielding a clear yellowish-brown oil ($n = 1.4816_D^{27}$) with a slight odour of the essential oil. After complete extraction of the free acids with ether, a yellow colouring matter remained in the aqueous phase. Part of this material was removed by shaking with chloroform, but it was not identified.

The hydrocarbon proved to be a low polymer. During saponification of the benzene extract and subsequent extraction with ether the residue that escaped collection after the addition of acetone was mechanically separated, washed, and dried (1.4 gm.). Evaporation of the ethereal solution of unsaponifiables left a soft brown resin, which was not further studied. The molecular weight of the polyisoprene was determined on a sample extracted from fresh gum (a) with 1% phenyl- β -naphthylamine in cold acetone, and (b) with 0.5% benzidine in nitrogen-saturated benzene. The material was not dried after acetone extraction. After precipitation from benzene it was dried to constant weight *in vacuo* at room temperature, and had a weight average molecular weight of about 19,000 (18).

Of the entire portion of the gum soluble in benzene, about 79% consisted therefore of impure polyisoprene, 15% was a viscous oil, and 6% was unsaponifiable matter. Solution of the unsaponifiables in ethanol left a small quantity of a colourless wax-like compound.

ETHANOL-BENZENE EXTRACT IV (see Diagram I)

Small quantities of fixed oil and unidentified substances were obtained from this extract. A soft brown resin (2.6 gm. amounting to 0.2% of the original dry gum), of which a considerable fraction was insoluble in ether, was obtained

on removal of the solvents. When collected, the matter suspended in ether formed a black tarry mass, from which nothing definite was obtained. Extraction of the ethereal solution with 5% sodium bicarbonate solution yielded a small quantity (0.18 gm.) of a viscous yellow oil ($n = 1.4930^{25}_D$). Subsequent extraction with sodium hydroxide solution (5%) yielded an even smaller quantity (0.04 gm.) of an odourless yellow oil. The ethereal solution remained sharply divided into three layers, which were treated separately. Removal of the clear dark brown bottom layer, and recovery of the solvent, left a brown resin (0.5 gm.) with a strong odour of the essential oil. The insoluble substances from the middle layer dried to a powder that gave a positive test with Fehling's solution, and on acidification yielded a yellow oil. Recovery of the solvent from the top layer left a yellowish waxy material that was precipitated from dioxane in an amorphous condition, and that gradually charred without melting when heated slowly to 300° C.

AQUEOUS EXTRACT V (see Diagram I)

Calcium tartrate, a dark tarry material yielding sugars after acid hydrolysis, soaps, and inorganic substances from the soluble ash components were found in the water extract. After seven consecutive extractions, each lasting for two days, there was no indication of an approaching end-point, and the extraction was discontinued. In this 14 day period, 10.7% of the original residue had been removed. The extracts, preserved under toluene, were filtered, and subsequently concentrated to a thin syrup.

The dry filter residue (5 gm.) was a light brown powder containing glistening scales and a small amount of calcium tartrate. A small amount (0.6 gm.) of this powder was boiled with 0.2 *N* potassium hydroxide solution, and the brown suspension filtered, acidified to pH 3, and extracted with ether. On re-extracting with 5% aqueous sodium bicarbonate, the entire extract passed to the aqueous phase. Removal of the ether left 0.06 gm. of a viscous yellow oil. About 10% of the filter residue therefore consists of free acids, held in the gum as soaps. Ignition of the material left after extraction with potassium hydroxide yielded 66% of ash, whereas the original filter residue contained 53.4% of ash.

The thin syrup (200 ml.) obtained by prolonged boiling of the filtrate (4 litres) was separated by filtration from masses of black tarry material and from a considerable inorganic residue containing calcium phosphate. The filtered liquor gave a positive test with Fehling's solution, but failed to form osazones. About 50 ml. of the filtrate was acidified to pH 3, and extracted several times with ether. Recovery of the solvent left a considerable quantity (1.5 gm.) of a dark oil ($n = 1.4882^{27}_D$), with a strong odour of the essential oil. After mild acid hydrolysis the residual liquor gave a heavy red precipitate with Fehling's solution and yielded a mixture of osazones that were not separately identified.

"INSOLUBLE" RESIDUE V (see Diagram I)

The residue contained 35.0% of lignin (16), 0.47% of nitrogen, and 40.1% of ash. Cellulose was identified microchemically.

Conclusion

The following approximate composition of the gum has been calculated from the above data.

	%
Fatty acid esters	50
α -Amyrin	26
Polyisoprene	6
Ash	4
Lignin	3
Cellulose	2
Protein ($N \times 6.25$)	0.8
Free fatty acids	0.2
Sterol	0.1
Resene	0.1
Essential oil	0.1
Calcium tartrate	Trace
Unidentified	7.7
	<hr/> 100.0

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PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

X. THE EFFECTS OF SURFACE-VOLUME RATIO AND REDUCED PRESSURE ON THE FERMENTATION OF CARBOHYDRATES BY *AEROBACILLUS POLYMYXA* AND *AEROBACTER AEROGENES*¹

BY G. A. ADAMS² AND J. D. LESLIE³

Abstract

The rate of fermentation of wheat mash by *Aerobacillus polymyxa* was markedly increased by providing a high surface-area-volume ratio. Exposure to air or oxygen *per se* was not a controlling factor since an atmosphere of nitrogen gave the same effect. Inhibition of fermentation of shallow layers of mash by exposure to carbon dioxide suggested that escape of fermentation gases (mainly carbon dioxide) might be the major factor affecting the fermentation rate. Support has been given to this hypothesis by the marked increase in rate obtained when wheat mash was fermented under reduced pressures.

Fermentation of 15% wheat mash, normally requiring 72 to 96 hr., were complete in 48 hr. under 5 in. pressure (absolute). The fermentation rate was accelerated by decreasing pressures but was retarded slightly by increasing depths of mash. The butanediol-ethanol ratio became progressively lower with decreasing pressures, showing that such conditions favour ethanol formation.

Fermentation of sugar media by *Aerobacter aerogenes* was only mildly stimulated by reduced pressures. This treatment compares unfavourably with aeration as a means of increasing the fermentation rate of this organism.

Introduction

The significance of surface-volume relations in the fermentation of wheat mash by *Aerobacillus polymyxa* was pointed out first by Katznelson (2). Shallow layers of whole wheat mash fermented at a faster rate and gave higher diol-ethanol ratios than deep layers. In spite of the importance of this observation it was not feasible to carry out large scale *A. polymyxa* fermentations in shallow layers. It became necessary to determine the factor or factors governing the fermentation rate and ratio of products under different surface-volume conditions and to find means of applying these factors to fermentation of deep mash by *A. polymyxa*. A less detailed investigation of a similar nature was also made of the *Aerobacter aerogenes* fermentation.

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Materials and Methods

In the experiments described in this paper, two strains of *Aerobacillus polymyxa*, N.R.C. C 4 (2) and C 3 (2) from our own collection, and *Aerobacter aerogenes* N.R.R.L. 199 were used.

For *Aerobacillus* fermentations 15% whole wheat mash containing 1% of calcium carbonate was used as medium. The medium for the *Aerobacter* fermentations consisted of 10% of dextrose, 0.09% of potassium mono-hydrogen phosphate, 0.09% of potassium dihydrogen phosphate, 0.025% of magnesium sulphate, and 0.2% of urea. The *Aerobacillus* inoculum was brought up in two 24-hr. stages on 5% whole-wheat-yeast-extract-calcium-carbonate; the medium for the *Aerobacter* inoculum was dextrose-corn-steep-liquor-calcium-carbonate. Inoculum equal to 3% of the volume of the mash was used. All fermentation media were incubated at 30° C. The methods of analyses for butanediol, ethanol, acetoin, and organic acids have been previously described (3).

Since the results in general with C 4 (2) and C 3 (2) were substantially the same, only those obtained with C 4 (2) are presented.

Experimental Results

Effect of Various Surface-volume Relations on the A. polymyxa Fermentation

The first experiments were designed to confirm the observation by Katznelson that the rate of fermentation of wheat mashes increased with larger surface-volume relations. Three-hundred-millilitre lots of 15% whole wheat mash were fermented in 500, 1000, 2000, 3000, 4000, and 6000 ml. Erlenmeyer flasks, using *A. polymyxa*. Mashes were analysed for diol and ethanol at 48, 72, and 96 hr. The experiment was done in triplicate.

The results given in Table I show that the rate of fermentation was greatly increased by a high surface-volume ratio, thereby confirming the original observation by Katznelson. The progressive increase in the diol-ethanol ratio from deep to shallow layers showed also that the rate of diol formation was enhanced more than that of ethanol. It may be noted also that all fermentations were practically complete at 72 hr. with the exception of that of the deepest mash.

The results for ethanol showed an optimum rate of formation at an intermediate depth of mash. This indicates that some factor besides surface-volume condition is involved, and it is suggested that the rate is subject to two opposing effects, an acceleration due to decreasing depth of mash and some inhibiting factor. It has been pointed out in another paper (1) that oxygen retards ethanol formation by *A. polymyxa*, and perhaps this accounts for the inhibition observed in mashes where surface exposure was relatively large. The main purpose of the present investigation was to determine the nature of the acceleration factor in the formation of both ethanol and diol.

In the foregoing experiment, the use of equal volumes of mash in different sized flasks permitted the surface area and depth of mash to vary simul-

TABLE I

EFFECT OF FERMENTING 300 ML. LOTS OF WHOLE WHEAT MASH WITH
A. polymyxa AT VARIOUS SURFACE-VOLUME RATIOS

Fermen- tation period, hr.	Surface, cm. ² Volume, ml.	Butanediol, %	Ethanol, %	Total products, %	Ratio
48	0.16	1.72	0.97	2.69	1.77
	0.49	2.42	1.25	3.67	1.93
	0.66	2.66	1.17	3.83	2.27
	0.86	2.85	1.22	4.07	2.34
	0.95	3.06	1.22	4.28	2.51
	1.32	3.19	1.17	4.36	2.73
72	0.16	2.19	1.18	3.27	1.85
	0.49	2.92	1.51	4.43	1.93
	0.66	3.14	1.37	4.51	2.29
	0.86	3.22	1.34	4.56	2.40
	0.95	3.26	1.19	4.45	2.74
	1.32	3.32	1.17	4.49	2.84
96	0.16	2.55	1.41	3.96	1.81
	0.49	3.11	1.56	4.67	1.99
	0.66	3.24	1.38	4.62	2.35
	0.86	3.28	1.31	4.59	2.50
	0.95	3.38	1.16	4.54	2.91
	1.32	3.37	1.09	4.46	3.09

taneously. By using 4-litre bottles instead of Erlenmeyer flasks, it was possible to keep the surface area constant and to vary the depth and volume. Fifteen per cent wheat mash was fermented at various depths in the bottles. Analyses for diol and ethanol were made as before at 24, 48, and 96 hr. The earlier period was included, as changes in the fermentation are most marked at this time; the 96 hr. analyses served to show the yield of total products at completion. It can be seen from the results in Table II that the rate of fermentation is closely related to mash depth and is greatly accelerated in shallow layers. It is clear that the beneficial effect was more marked on diol than on ethanol formation, but since surface area was held constant the effect of mash depth on ethanol was more pronounced than in the previous experiment.

The deeper the mash layer and consequently the more anaerobic the conditions the higher was the final proportion of ethanol formed. The slight drop in ethanol formation between 48 and 96 hr. in the shallowest layer cannot be satisfactorily explained, but may be due to either loss by evaporation or conversion to organic acids. The fact that the theoretical yield of products was obtained in the shallow fermentations showed that there was no shift from fermentative to oxidative dissimulation.

The previous experiments were carried out in air and it remained to be shown whether or not air (oxygen) *per se* was a factor in increasing fermentation rate. The simplest means of throwing light on this point was to do the same experiment in an anaerobic atmosphere, e.g., nitrogen.

TABLE II

EFFECT OF FERMENTING WHOLE WHEAT MASH WITH *A. polymyxa* UNDER CONDITIONS OF CONSTANT SURFACE, 227 CM.², AND VARYING DEPTH

Fermen- tation period, hr.	Surface, cm. ² Depth, cm.	Butanediol, %	Ethanol, %	Total products, %	Ratio
24	344	1.44	0.57	2.01	2.53
	151	1.27	0.59	1.86	2.15
	87	0.85	0.44	1.29	1.93
	60	0.83	0.47	1.30	1.77
	43	0.76	0.41	1.17	1.85
48	344	3.02	1.35	4.37	2.24
	151	2.72	1.44	4.16	1.89
	87	2.07	1.09	3.16	1.90
	60	1.67	0.92	2.59	1.82
	43	1.50	0.83	2.33	1.81
96	344	3.29	1.26	4.55	2.61
	151	3.06	1.47	4.53	2.08
	87	2.85	1.75	4.60	1.63
	60	2.54	1.57	4.08	1.60
	43	2.10	1.13	3.23	1.86

The effect of nitrogen on the fermentation as compared with that of air was studied by fermenting 300-ml. lots of wheat mash in 500, 2000, and 6000 ml. flasks. Nitrogen was passed over the surface of the media. A set of control mashes was fermented under identical conditions except that they were allowed to stand in their own gaseous atmospheres. Analyses for diol and ethanol were made at 24, 48, and 72 hr. The results are shown in Table III. In so far as total products are concerned, fermentation in shallow layers is markedly increased by exposure to nitrogen as well as to air, but the distribution of diol and ethanol is different in the two gases. In general, air

TABLE III

EFFECT OF FERMENTING 300 ML. OF WHOLE WHEAT MASH WITH *A. polymyxa* UNDER AEROBIC AND ANAEROBIC CONDITIONS AT VARIOUS SURFACE-VOLUME RATIOS

Fermen- tation period, hr.	Surface, cm. ² Volume, ml.	Butanediol, %		Ethanol, %		Total products, %		Ratio	
		Air	Nitrogen	Air	Nitrogen	Air	Nitrogen	Air	Nitrogen
24	0.16	0.78	0.93	0.41	0.53	1.19	1.46	1.90	1.75
	0.66	1.53	1.36	0.65	0.83	2.18	2.19	2.35	1.62
	1.32	1.96	1.62	0.76	1.07	2.72	2.69	2.58	1.31
48	0.16	1.96	1.62	0.84	1.26	2.80	2.88	2.33	1.23
	0.66	2.75	2.54	1.38	1.77	4.13	4.31	2.00	1.44
	1.32	2.93	2.66	1.33	1.76	4.26	4.42	2.20	1.51
72	0.16	2.22	2.03	1.17	1.26	3.39	3.29	1.90	1.61
	0.66	2.93	2.54	1.39	1.77	4.32	4.31	2.11	1.44
	1.32	3.06	2.66	1.22	1.76	4.28	4.42	3.06	1.51

stimulated the production of diol more than did nitrogen, but the effect on ethanol formation was the reverse. It may be concluded, therefore, that the accelerated rate of fermentation in shallow layers is not related to exposure to air *per se* since a nitrogen atmosphere gives a similar effect. It seemed likely, therefore, that the accelerating factor must involve some characteristic that shallow layers of mash possess under both aerobic and anaerobic conditions. This suggested that large surface area and relatively small depth would provide for more rapid escape of the gases (carbon dioxide and hydrogen) produced during fermentation and that this escape of gases might be the factor affecting the rate.

If the hypothesis stated above is correct then exposure of mashes of varying depths to an atmosphere of the fermentation gases should give an approximately constant fermentation rate. Since carbon dioxide makes up the main bulk of these gases its effect on the fermentation rate in various depths of mash was investigated.

As previously described, 15% mash was fermented at varying depths in 4-litre bottles in a changing atmosphere of carbon dioxide. Mashes fermented in their own atmospheres provided controls. The results given in Table IV show that at all depths mash fermented at practically the same rate under carbon dioxide. The data for total products show also that in the shallowest layer of mash the rate of fermentation was slightly depressed compared to the rate in air.

TABLE IV

EFFECT OF CONSTANT SURFACE, 227 CM.², AND VARYING DEPTH ON THE FERMENTATION OF 15% WHOLE WHEAT MASHES BY *A. polymyxa* UNDER AIR AND CARBON DIOXIDE ATMOSPHERES

Fermentation period, hr	Surface, cm. ² Depth, cm	Butanediol, %		Ethanol, %		Total products, %		Ratio	
		Air	Carbon dioxide	Air	Carbon dioxide	Air	Carbon dioxide	Air	Carbon dioxide
24	151	1 21	0 90	0 65	0 46	1 86	1 36	1 86	1 96
	87	1 06	0 91	0 51	0 49	1 57	1 40	2 12	1 86
	43	0 90	0 91	0 53	0 50	1 43	1 40	1 70	1 82
48	151	2 57	2 08	1 28	1 07	3 85	3 15	2 01	1 94
	87	2 25	2 10	1 21	1 22	3 46	3 32	1 86	1 72
	43	1 94	2 10	1 12	1 24	3 16	3 34	1 73	1 69
72	151	3 07	2 74	1 61	1 39	4 68	4 13	1 91	1 96
	87	2 89	2 77	1 68	1 80	4 57	4 57	1 72	1 54
	43	2 62	2 76	1 63	1 81	4 25	4 57	1 61	1 52

These experiments indicate that rapid fermentation of wheat mashes in thin layers, or, more precisely, under conditions of large surface-volume ratio, is attributable to the rapid escape of carbon dioxide. It might be contended that under conditions of active fermentation any mash would be covered by carbon dioxide, which would impose the same conditions as employed in the above experiment. Quantitatively, however, the atmosphere above a fer-

menting mash exposed to air cannot be 100% carbon dioxide since some air will reach the mash surface by diffusion. It is obvious, then, that in mashes of small surface-volume ratio the concentration of carbon dioxide will be relatively high, in which case the conditions will approximate more closely those of the above experiment.

Since the ready escape of carbon dioxide from fermenting mashes appeared to be an important factor in accelerating the rate of fermentation, it seemed logical to suppose that decreasing the total gas pressure over the mash would help achieve this effect. Furthermore such treatment would be applicable to deep as well as to shallow mashes.

Effect of Reduced Pressure on Fermentation Rate

A 2000 ml. flask was fitted with a water-cooled reflux condenser, which in turn was connected to a vacuum pump through a -80°C . cold trap and a mercury manometer. One litre of 15% whole wheat mash, inoculated with *A. polymyxa*, was put in the flask and the pressure reduced to 5 in. of mercury absolute. As a control an identical fermentation was carried out at atmospheric pressure. No foaming difficulties were encountered under reduced pressure. Samples were removed from control and test flasks at 24, 36, 48, and 96 hr. and analysed for diol and ethanol. The results are given in Table V. The effect of reduced pressure was to increase greatly the rate of fermentation, and the formation of both diol and ethanol. The relatively low diol-ethanol ratio indicated that the effect was more pronounced on ethanol. This experiment offers substantial proof of the hypothesis that removal of carbon dioxide is a primary factor in increasing the fermentation rate.

TABLE V

THE EFFECT OF REDUCED PRESSURE ON RATE OF FERMENTATION OF WHEAT MASH BY *A. polymyxa*

Ferment- ation time, hr	Pressure, in Hg	Butanediol, %	Ethanol, %	Total products, %	Ratio
24	30	1 03	0 50	1 53	2 06
	5	2 00	1 44	3 44	1 39
36	30	1 55	0 79	2 34	1 96
	5	2 40	1 85	4 25	1 30
48	30	2 09	1 13	3 22	1 85
	5	2 45	1 94	4 39	1 26
96	30	3 00	1 49	4 49	2 02
	5	2 45	2 02	4 47	1 21

In a further experiment 2-litre lots of 15% wheat mash were fermented under 10 in. of mercury absolute pressure in 4-litre Pyrex bottles. Control mashes were fermented at atmospheric pressure under similar conditions. Analyses for diol, ethanol, and pH were made at 24, 48, 72, 96, and 120 hr. These intervals provided a measure of the rate of fermentation as well as an

indication as to whether or not total products are realized under reduced pressure treatments. The results of duplicate mashings are given in Table VI. Again it is apparent that reduced pressure markedly accelerated the fermentation rate, the reactions under these conditions being about 95% complete in 48 hr. as compared with 60% completion in the control fermentations. A theoretical yield of total products (diol + ethanol = 4.6%) was obtained,

TABLE VI
EFFECT OF REDUCED PRESSURE ON RATE OF FERMENTATION AND
PRODUCT YIELD BY *A. polymyxa*

Fermen- tation time, hr.	Pressure, in. Hg.	Butanediol, %	Ethanol, %	Total products, %	Ratio	pH
24	30	0.85	0.51	1.36	1.67	6.17
	10	1.66	1.14	2.80	1.45	5.78
48	30	1.76	1.03	2.79	1.71	6.00
	10	2.44	1.92	4.36	1.27	5.80
72	30	2.47	1.52	3.99	1.62	5.95
	10	2.51	1.96	4.47	1.28	5.95
96	30	2.65	1.73	4.38	1.53	5.90
	10	2.52	2.02	4.45	1.25	5.97
120	30	2.76	1.78	4.54	1.55	5.85
	10	2.52	2.06	4.58	1.23	5.98

showing that reduced pressure did not interfere with normal fermentative dissimulation. The pH changes were also worthy of note. Under atmospheric conditions there was a normal progressive fall in pH throughout the course of the fermentation. On the other hand, under reduced pressure the pH of the mash dropped to a level of 5.78 in 24 hr. and thereafter rose progressively to 5.98 in 120 hr. This rise in pH suggested proteolytic breakdown of the wheat proteins to give ammonia, and also that such decomposition only occurs to an appreciable extent when the carbohydrate source is nearly all consumed.

Effect of Depth of Mash and Absolute Pressure on the Fermentation Rate

Since acceleration of the fermentation rate by reduced pressure seemed to be due largely to the removal of fermentation gases it was important to know the effectiveness of the treatment at various depths of mash. It appeared likely that the removal of gases would become more difficult with increasing depth. Within the limits imposed by laboratory equipment an investigation was made of the interaction of mash depth and pressure on the rate of fermentation.

Fifteen per cent whole wheat mashes were fermented with *A. polymyxa* in 4-litre Pyrex bottles fitted with reflux condensers as previously described. The mash depths were 1.5, 2.6, 4.8, 8.8, and 13.2 cm. The pressures selected for study were 20, 10, and 5 in. of mercury absolute. Controls consisted of similar mashes of the same depths fermented at atmospheric

pressure. The analyses for diol and ethanol were made at 24 hr. only, as previous experiments had shown that the effect of reduced pressure was most marked at that time. Since considerable difficulty was experienced in obtaining concordant results from one experiment to another, even in the controls, a series of five experiments with controls was carried out at each pressure. The average results of the analyses from all experiments judged to be satisfactory are given in Table VII.

TABLE VII

EFFECT OF REDUCED PRESSURE AT VARIOUS DEPTHS AT 24 HR. ON THE FERMENTATION OF WHEAT BY *A. polymyxa*

Depth, cm.	Butanediol, %				Ethanol, %				Total products, %				Diol-ethanol ratio			
	Pressure, in. Hg				Pressure, in. Hg				Pressure, in. Hg				Pressure, in. Hg			
	30	20	10	5	30	20	10	5	30	20	10	5	30	20	10	5
1.5	1.06	1.29	1.63	2.00	0.48	0.68	1.09	1.49	1.54	1.97	2.72	3.49	2.21	1.90	1.50	1.34
2.6	0.89	1.14	1.60	1.43	0.46	0.65	1.07	1.39	1.35	1.79	2.67	3.32	1.93	1.75	1.50	1.39
4.8	0.76	1.03	1.65	1.85	0.42	0.61	1.16	1.52	1.18	1.64	2.81	3.37	1.81	1.69	1.42	1.22
8.8	0.73	1.05	1.60	1.82	0.43	0.66	1.12	1.48	1.16	1.71	2.72	3.32	1.70	1.59	1.42	1.23
13.2	0.72	1.12	1.67	1.83	0.44	0.72	1.23	1.60	1.16	1.84	2.90	3.43	1.64	1.55	1.36	1.14

At all depths of mash there was a clear-cut effect of reduced pressure on the yields of diol and ethanol; both were markedly increased by progressive reduction in pressure. However, the effect was more marked on ethanol than on diol as shown by the progressive drop in the diol-ethanol ratio with decreasing pressure. The significance of these results was confirmed by statistical analysis. As shown before, under atmospheric pressure the overall effect of decreasing the mash depth was to increase the rate of formation of products, diol formation being accelerated more than that of ethanol. At 20 in. pressure the effect of depth on diol formation was similar to that at atmospheric pressure, with the exception of the deepest mash. At the lower pressures the effect of depth was less marked owing perhaps to the more effective removal of carbon dioxide. The increased production of ethanol in deep mashes had been shown earlier to be related to the degree of anaerobiosis; lower pressures accentuate this condition by decreasing the oxygen content of the gas atmosphere.

At all three pressures below atmospheric the minimum yield of total products occurred at some depth intermediate between the two extremes. This effect applied to both diol and ethanol, and may have been due to a mixing phenomenon that became effective at reduced pressures. It was noted that bubbles of gas floated bran and other solid particles of the media to the surface where gas release permitted the solids to drop back slowly to the bottom of the flask. In this way, a steady and thorough mixing of the mash took place, which appeared to aid considerably in the evolution of gas. Since this mixing was more pronounced in deep mashes the net effect was to partially neutralize

the acceleration normally observed in shallow mashers. The mash depths used in these experiments were chosen arbitrarily and because of experimental conditions were not great compared to those found in large scale operations. It seems reasonable to suppose that if depth of mash were increased greatly it would eventually become a dominant factor in the release of fermentation gases. Under such conditions agitation might be combined with reduced pressure to advantage.

Effect of Reduced Pressure on the Fermentation of Glucose by Aerobacter aerogenes

In view of the foregoing results, which showed an accelerating effect of reduced pressure on the rate of fermentation of wheat mash by *Aerobacillus polymyxa*, it seemed worthwhile to subject *Aerobacter aerogenes* fermentations to the same treatment. The culture media used in this experiment has been described earlier in the paper. Quantities of media were put in 4-litre bottles to give depths of 1.5, 2.6, 4.8, 8.8, and 13.2 cm., respectively. The bottles were fitted with water-cooled reflux condensers and connected to a vacuum pump as before. Absolute pressures of 20, 10, and 5 in. of mercury were used in the experiment. Controls for each reduced pressure experiment were provided by similar media fermented at atmospheric pressure. Analyses were made for butanediol, ethanol, and acetoin at the end of a 24 hr. fermentation period. The diol figure used in calculating the diol-ethanol ratio included acetoin. Results are given in Table VIII.

TABLE VIII

EFFECT OF REDUCED PRESSURE, AT 24 HR., ON THE FERMENTATION OF GLUCOSE AT VARIOUS DEPTHS BY *Aerobacter Aerogenes* N.R.R.L. 199

Depth of media, cm	Butanediol, %				Ethanol, %				Acetoin, %				Diol-ethanol ratio			
	Pressure in Hg				Pressure, in Hg				Pressure in Hg				Pressure, in Hg			
	30	20	10	5	30	20	10	5	30	20	10	5	30	20	10	5
1.5	0.36	0.50	0.58	0.61	0.24	0.32	0.37	0.36	0.04	0.06	0.05	0.04	1.50	1.56	1.57	1.69
2.6	0.31	0.47	0.54	0.68	0.21	0.26	0.36	0.39	0.03	0.06	0.06	0.06	1.47	1.80	1.50	1.77
4.8	0.30	0.36	0.46	0.74	0.20	0.25	0.34	0.43	0.03	0.06	0.05	0.06	1.50	1.44	1.35	1.72
8.8	0.29	0.35	0.56	1.21	0.19	0.19	0.37	0.69	0.02	0.05	0.05	0.10	1.52	1.54	1.51	1.76
13.2	0.29	0.32	0.60	1.36	0.18	0.21	0.37	0.78	0.02	0.04	0.06	0.10	1.61	1.52	1.62	1.75

Increasing reduction of pressure produced a progressive increase in yields of both diol and ethanol in all depths of medium. The effect of increasing depth at atmospheric and 20 in. pressure was to decrease the rate of fermentation. Ten inches pressure at an intermediate depth gave the minimum rate, while at the lowest pressure (5 in.) the deep mashers fermented much more rapidly.

These results parallel, in general, those obtained in the *Aerobacillus polymyxa* fermentations, but there is one outstanding difference, namely, the reversed effect of depth at the lowest pressure. Apart from inherent differences in

the two types of organism, this effect may be attributed to the much lower viscosity of the glucose medium, which permitted greater mixing and more ready escape of gas with increasing depth.

Discussion

Provision for the rapid removal of fermentation gases from whole wheat mash increased the rate of fermentation by *A. polymyxa*. Further confirmation was provided in previous work by aeration of fermenting media with various gases (1). Although oxygen and air specifically stimulated diol formation, and nitrogen and hydrogen had a similar effect on ethanol production, the over-all effect of aeration was to increase the rate of fermentation. The effect common to all the aeration treatments was the mechanical removal or sweeping out of the carbon dioxide produced by fermentation. The fact that aeration produced the same general effect as reduced pressure indicated that the latter treatment did not bring about any special metabolic activity of the organism.

It was noted during the course of the present experiments with reduced pressure as well as in previous investigations involving aeration that all treatments caused a marked increase in bacterial numbers. Although this was to be expected with air and oxygen it was not anticipated with nitrogen and hydrogen nor with the reduced pressure treatments. The increased fermentation rate might be explained on the basis of greater numbers of bacteria although as yet no satisfactory correlation between numbers and products yield has been established. Results of further investigations on this point will be published shortly.

The effect of reduced pressure on *Aerobacter* fermentations was not as marked as on *Aerobacillus* as shown by the yield of total products in 24 hr. It is known, however, that rapid fermentation with *Aerobacter* cannot be achieved without vigorous aeration; normally this is accomplished with air but it has been shown that nitrogen is equally effective (1). This observation was interpreted as meaning that removal of carbon dioxide by the aerating gas was the main factor influencing rate of fermentation. However, a similar removal of gas by reduced pressure and the establishment of anaerobic conditions (as under nitrogen) failed to give the same stimulation to fermentation, especially in the shallower layers of media. Only at the lowest pressure and in the deepest media did the *Aerobacter* fermentation give markedly increased yields.

Acknowledgment

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PACKAGING

III. EFFECT OF MOULD GROWTH AND AGEING ON THE WATER-VAPOUR TRANSMISSION OF PACKAGING MATERIALS¹

BY C. G. LAVERS² AND W. I. ILLMAN³

Abstract

Packaging materials were dusted with mould spores and stored in a cabinet at 95° F. and 95 to 100% relative humidity for periods of one to eight weeks.

M.S.T. and M.S.A.T. "Cellophane" were attacked only slightly by mould, but deterioration of the heat-sealing, moisture-proof lacquer occurred during storage under conditions suitable for optimum mould growth. Moulds grew abundantly on M.S.Y.T. Cellophane. Wax-coated materials supported abundant mould growth, and their water-vapour transmission values increased when wax peeled from the surface of the sheet. The transmission rate of laminated materials having metal foil as one layer was not greatly affected by mould growth or delamination of the other layers. Abundant mould growth developed on most samples of kraft, and on glassine. Very little mould developed on cellulose acetate, Pliofilm, or vinyl-film.

Introduction

The effect of mould growth is undoubtedly an important factor in the deterioration of packaging materials if the atmosphere surrounding the package is of high humidity, or if the package contains material of high moisture content, i.e., material likely to be in equilibrium with high humidities. Concurrently with exposure to conditions favourable to the growth of moulds, changes may occur in packaging materials such as weakening of the fibre structure, delamination, or separation of a coating from the base material. Hence, the present investigation was undertaken to assess the effects of mould growth and ageing on the water-vapour transmission of a variety of packaging materials.

Materials and Methods

The materials used were various combinations of kraft paper, metal foil, glassine, "Cellophane," cellulose acetate, vinyl-film, and Pliofilm, both waxed and unwaxed. In addition, different grades and plies of Cellophane were investigated. When samples of duplex or triplex Cellophane were tested, the layers were heat sealed around the edge of the samples since these materials would be sealed in this manner when in use. Details of materials are noted in Table I.

The initial water-vapour transmission of the materials was determined. Samples were then dusted with mould spores of a variety of species and placed in a mould infested cabinet, operating at 95° F. and at 95 to 100% relative

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humidity. Species included in the inoculum were; *Syncephalastrum racemosum*, *Paecilomyces variota*, *Penicillium* spp., *Aspergillus niger*, *Aspergillus flavus*, *Aspergillus terreus*, *Chaetomium globosum*, and *Stachybotrys atra*. Samples were removed after storage for one, two, four, and eight weeks, were inspected for mould growth and physical deterioration, and the water-vapour transmission determined. All measurements of water-vapour transmission were made using a vapometer at a vapour pressure differential of approximately 26 mm. of mercury and a dry bulb temperature of 80° F. The vapometer was the same as that used in a previous study of packaging materials (2). It consisted of a flanged aluminium cup containing 10 ml. of water. Over the mouth of the cup the sample could be attached by another flange securely bolted to effect the seal. Although more accurate methods of measurement are desirable (1), this technique was satisfactory for the present purpose and provided a relative scale for comparing changes in water-vapour transmission occasioned by mould growth and ageing.

Results

The results are shown in Figs. 1 to 20, in which the figure numbers correspond with the material numbers given in Table I. All water-vapour transmissions plotted are averages of triplicate determinations. The severity of attack by moulds was classified into four groups: none (*N*), surface free of mould growth; slight (*S*), growth had started at particles of foreign matter on the surface of the material; medium (*M*), growth was more general and moulds were actually feeding on the packaging material; abundant (*A*), growth was heavy and general.

M.S.T. and M.S.A.T. Cellophane were only slightly attacked by moulds except in spots where the lacquer had been damaged by heat sealing (Figs. 1, 2, 3, and 5). It is believed that the fairly large increase in the water-vapour transmission of these materials was due to loosening and cracking of the heat-sealing lacquer brought about by the severe conditions of storage, rather than to mould growth. In contrast to the above grades, M.S.Y.T. Cellophane (Fig. 4) developed an abundant growth of mould over its entire surface. Possibly a difference in the processing of this type of film caused this greater proliferation of organisms. There was a much smaller increase in the transmission of duplex and triplex Cellophanes (Figs. 2, 3, 4, and 5) than in that of the single sheet (Fig. 1). This probably occurred because some protection was afforded the lacquer on the inner faces of the Cellophane.

Abundant mould growth occurred on all materials that were wax-impregnated or wax-coated (Figs. 6 to 10). Wax-impregnated kraft (Fig. 6) showed a large increase in water-vapour transmission concurrent with abundant mould development. Wax-coated kraft (Fig. 7) suffered only a slight increase in water-vapour transmission, and only a slight separation of the wax from the kraft. Wax-coated Cellophanes (Figs. 8 and 9), however, showed large increases in their water-vapour transmission, and the wax tended to peel from the Cellophane. The increased transmission values of wax-coated

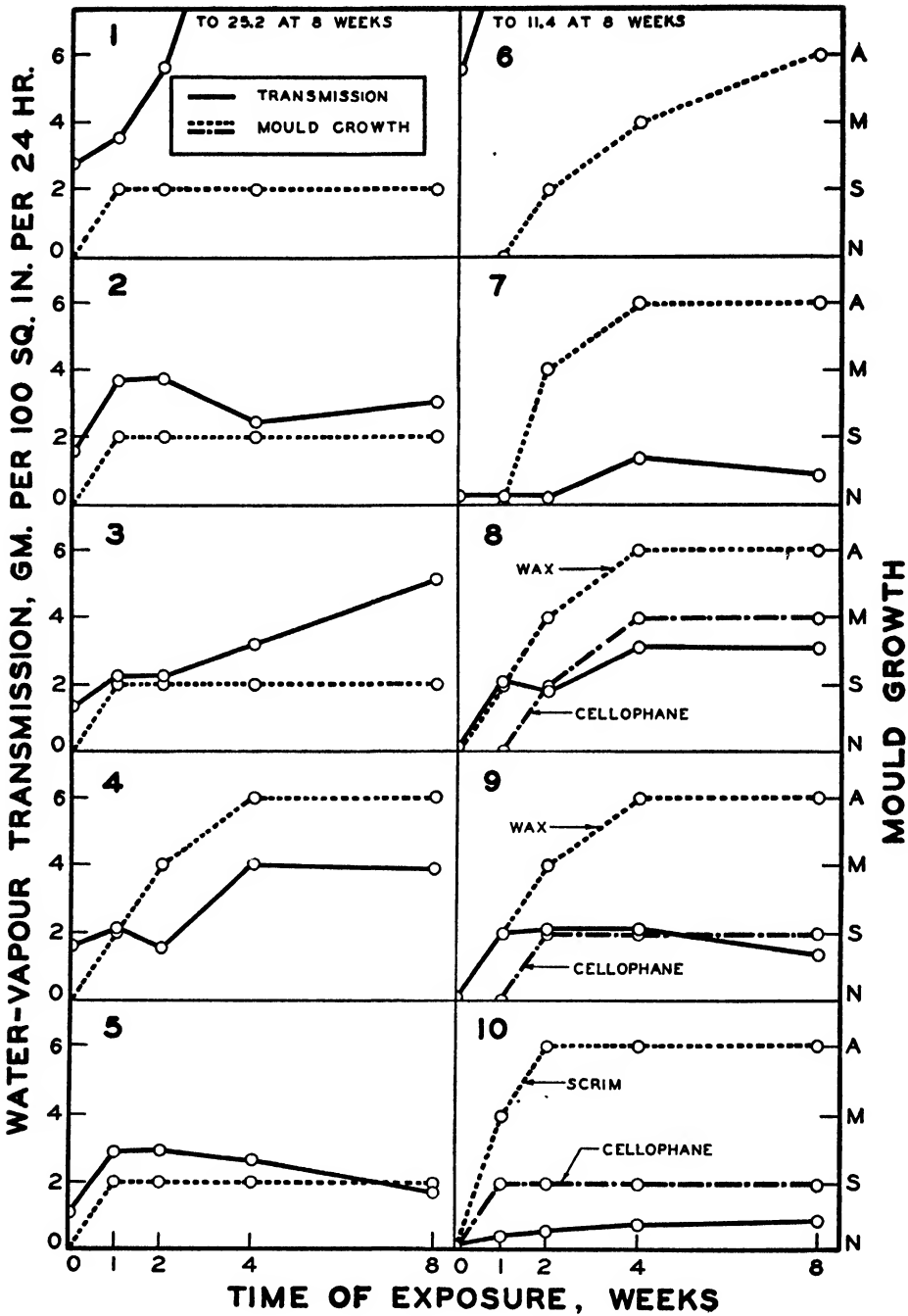
TABLE I
PACKAGING MATERIALS STUDIED

Material No.	Description of material
	Cellophanes
1	300 M.S.A.T., single
2	300 M.S.T., duplex
3	300 M.S.A.T., duplex
4	300 M.S.Y.T., duplex
5	300 M.S.A.T., triplex
	Waxed materials
6	40 Lb. kraft, wax impregnated
7	40 Lb. wet strength kraft, wax coated* 40 lb./ream
8	300 M.S.T. Cellophane, wax coated* 40 lb./ream
9	300 M.S.T. Cellophane laminated to 300 M.S.T. Cellophane, wax coated* 40 lb./ream
10	300 M.S.A.T. Cellophane laminated to scrim. Cellophane lightly waxed, scrim heavily waxed*
	Laminated materials
11	300 M.S.T. Cellophane laminated to 300 M.S.T. Cellophane
12	300 M.S.T. Cellophane laminated to metal foil
13	300 M.S.A.T. Cellophane laminated to 25 lb. kraft.
14	40 Lb. kraft laminated to alloyed lead foil laminated to 300 M.S.T. Cellophane
15	Scrim laminated to kraft laminated to alloyed lead foil with butvar coating
16	25 Lb. kraft laminated to cellulose acetate
17	25 Lb. kraft laminated to 25 lb. bleached glassine
18	25 Lb. bleached glassine laminated to 25 lb. bleached glassine
19	Vinyl-film laminated to both sides of 0.001 in. aluminium foil
	Pliofilm
20	Plioilm, single

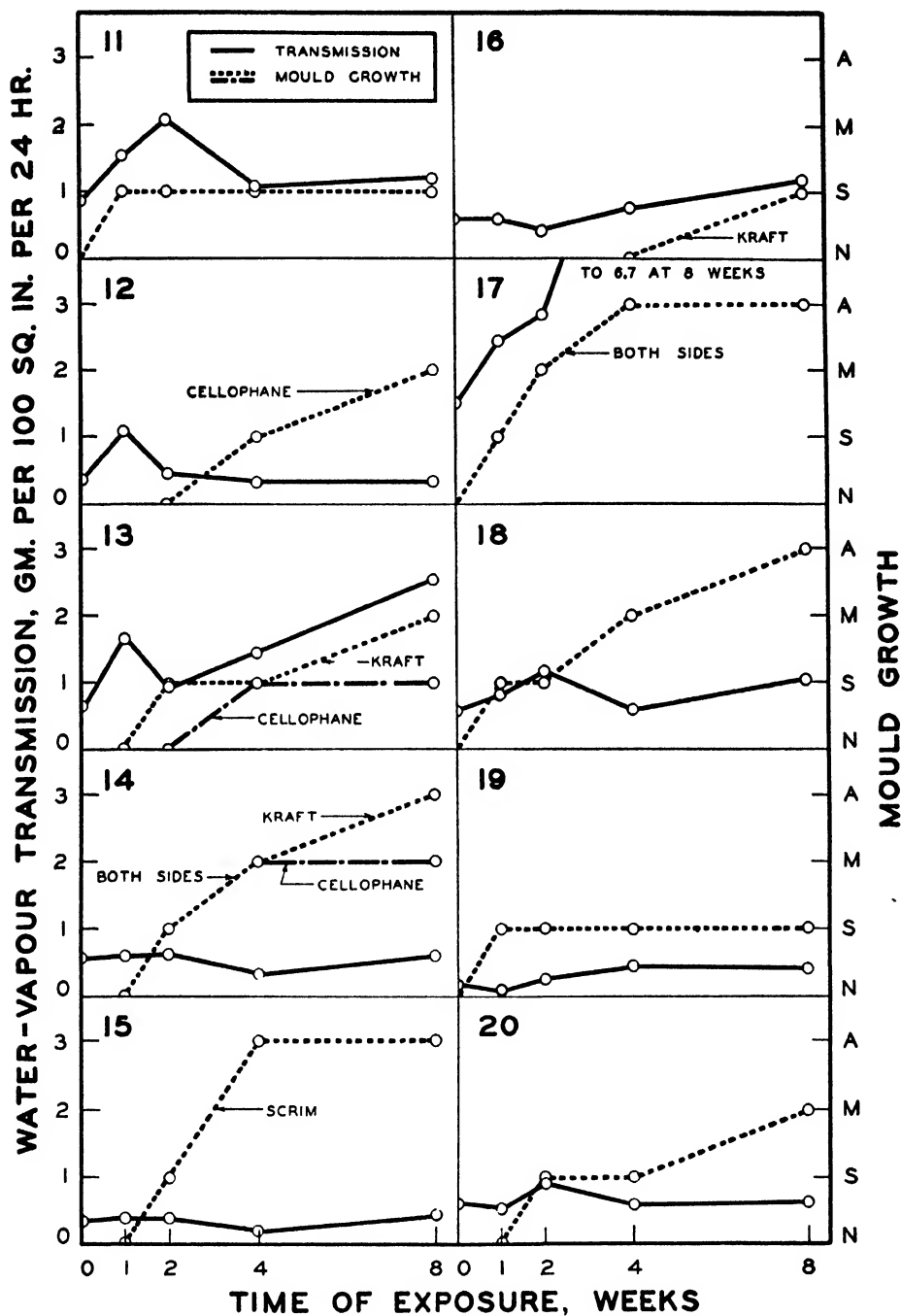
* Flexible wax compound.

materials were probably the result of this loosening of the wax coating. The data show the extent of the reduction in water-vapour transmission resulting from wax application, and its subsequent increase with separation of the wax from the base stock. Scrim laminated to Cellophane and heavily waxed (Fig. 10) supported abundant mould growth and showed a gradual but consistent increase in water-vapour transmission.

The water-vapour transmission of laminated materials having metal foil as one layer was not greatly affected even though moulds grew abundantly on the other layers and considerable delamination occurred. Examples of this were: Cellophane laminated to metal foil (Fig. 12), where considerable delamination occurred, and kraft laminated to foil laminated to Cellophane (Fig. 14), where considerable delamination of the Cellophane and slight delamination of the kraft occurred. Also, the foil blistered slightly on samples of foil laminated to kraft backed with scrim (Fig. 15). Very little mould grew on vinyl-film laminated to aluminium foil (Fig. 19) and very slight delamination occurred. This very low increase in water-vapour transmission indicated the general desirability of using a foil type barrier when



FIGS. 1-10. Effect of mould growth and ageing on the water-vapour transmission of Cellophane and of waxed packaging materials. (See Table I for key).



FIGS. 11-20. Effect of mould growth and ageing on the water-vapour transmission of laminated packaging materials and of Pliofilm. (See Table I for key).

severe conditions of temperature and humidity are likely to be encountered. Equal protection might not be provided, however, in a completed package, since the barrier would be of little value if the material forming the heat-sealing surface loosened from the foil.

Most of the materials having kraft paper as an outer layer developed abundant mould growth on the kraft side. In addition to supporting a heavy growth of moulds on the kraft side, samples of Cellophane laminated to kraft (Fig. 13) delaminated considerably and the water-vapour transmission showed a significant increase. No mould grew on the cellulose acetate sheet laminated to kraft (Fig. 16), and only a slight amount on the kraft. The acetate sheet appears to have inhibited growth on the kraft. Moulds grew abundantly on kraft laminated to glassine (Fig. 17) and caused marked deterioration, penetrating the sheet completely at one point. Laminated glassine itself (Fig. 18) supported abundant mould growth, but the increase in water-vapour transmission was much less than that of glassine laminated to kraft.

Pliofilm (Fig. 20) showed only slight mould growth, and little increase in water-vapour transmission.

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DRIED MILK POWDER

Y. THE PHOTOLYSIS OF RIBOFLAVIN IN MILK POWDERS¹

BY W. A. BRYCE²

Abstract

Exposure of milk powders to sunlight resulted in a much greater destruction of riboflavin than did exposure to ultra-violet light in the range 3200 to 4200 Å. The rate of photolysis was greater for skim-milk powders than for whole milk powders. Increased intensities of visible light accelerated riboflavin destruction. In the spectral region of 4200 to 5600 Å the wave band causing the greatest destruction in liquid skim-milk had a principal wave-length of 4450 Å, which corresponded to a maximum in the absorption spectrum of riboflavin. The rate of photolysis of riboflavin was a function of both wave-length and intensity of the impinging energy.

Introduction

Riboflavin, or vitamin B₂, is a valuable component of foodstuffs. It is effective in promoting growth in rats, and in preventing lesions of cheilosis and nutritional anemia in humans (11). It is also an important factor in cell respiration and metabolism (11). It is stable to heat, but is readily decomposed when exposed to light (4). Since milk is an important source of riboflavin, the photochemical decomposition, or photolysis, of this vitamin in milk has been studied. Liquid whole milk in commercial milk bottles exposed to summer sunlight for two hours lost two-thirds of its riboflavin (6, 9, 15). Sunlight apparently causes much greater destruction of riboflavin than ultra-violet light, as it has been reported that commercial irradiation of milk sufficient to produce a vitamin D content of 400 U.S.P. units per quart had no effect on the riboflavin content (3). Another investigator reported a slight decrease in riboflavin content of milk as a result of commercial irradiation (16).

Increases in either alkalinity or temperature accelerate the photolysis of riboflavin in liquid whole milk. Temperature and pH are believed to affect the photodecomposition rather than other chemical decompositions, for when riboflavin solutions are heated in the dark at 100° C. under mild acid conditions for four hours, little or no destruction of the vitamin occurs (14).

Since milk powder may be exposed to light during post-drying handling and storage and while it is being prepared for consumption, it was felt that some information on the photolysis of riboflavin in milk powders would be of value. This paper describes an investigation designed to study the effect of sunlight and ultra-violet light on the riboflavin content of milk powders of various fat contents obtained from different plants. It also includes some incidental measurements on riboflavin destruction in liquid skim-milk and on light absorption by riboflavin.

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² Biochemist, Food Investigations.

Materials and Methods

The powders used in the study were the commercial spray-dried products of two Canadian companies. Powders of 1, 26, 28, and 30% butter fat were obtained from one plant, and of 1, 26, and 28% butter fat from another, all powders having a moisture content of 3.0%.

All riboflavin determinations were made using the photofluorometric method developed by Hodson and Norris (5). After the initial riboflavin contents were determined, samples of the powders were placed in sealed Pyrex glass containers, and were analysed for riboflavin content after exposure to sunlight for 60, 110, and 215 hr. (the latter period corresponding to 48 days' storage) at an average temperature of 6.1° C. (43° F.). The average light energy falling on the powders was assumed to be 1.8 cal. per sq. cm. per min. During the study the containers were shaken by hand every three days in an attempt to ensure that all of the powder would be exposed. Control samples of the powders were stored in light-proof containers at the same temperature.

Additional samples of the same powders were exposed in Pyrex glass containers at a distance of 6 in. from a 250 watt Purple-X bulb that emitted ultra-violet light in the range 3200 to 4200 Å. The energy falling on the samples was calculated to be approximately 0.0034 cal. per sq. cm. per min. Riboflavin determinations were made after periods of 75, 150, and 300 hr. (42 days' storage). The temperature during this phase of the study was 38° C. (100° F.). The powders were shaken after each 10 hr. of exposure. Control samples in light-proof containers were stored under the same conditions.

The effect of variations in 'light' intensity on the rate of photolysis of riboflavin in milk powders was also studied, as calculations on the energies falling on the samples from the sun and from the ultra-violet bulb showed great differences between the two sources. Samples of whole and skim-milk powders were sealed in flat glass containers, and were exposed for 75 hr. at distances of 1 ft. and 4 ft. from a 100 watt tungsten lamp. The energy intensities at the two levels were 0.0036 and 0.0004 cal. per sq. cm. per min., respectively. The destruction of the riboflavin at each intensity of light was determined.

It was recognized that the riboflavin destruction caused by different parts of the near ultra-violet and visible spectrum might not be equal, and therefore an attempt was made to determine which bands were the most destructive. Samples of fresh skim-milk in glass cuvettes were exposed to successive wave bands from 4200 to 5600 Å, all bands being 200 Å wide and of equal intensity. The bands were obtained by the use of Corning glass filters. The intensities, measured by means of a Weston exposure meter, were adjusted to a constant value by varying the potential applied to the tungsten lamp used as the light source. After exposure for four hours the riboflavin content of the milk samples was determined.

The absorption spectrum of riboflavin in aqueous solution was also determined for the above light range, as it was believed that a direct relation

should exist between the wave-lengths causing maximum destruction and those at which the greatest absorption occurred. A Beckman Quartz Spectrophotometer was used to determine the absorption spectrum.

Control samples of powders in light-proof containers were maintained under comparable storage conditions whenever powders were exposed to visible or ultra-violet light. No decrease in riboflavin content occurred in any of these control samples during storage.

Results

The data for whole and for skim-milk powders were assessed by analysis of variance. No difference was observed between samples of whole or between samples of skim powders exposed to sunlight. The time of exposure had an effect on the whole milk powders but any change in skim powders was not assessed as significant. The results for samples exposed to sunlight are shown in Fig. 1. Marked destruction of the riboflavin occurred during the first 60

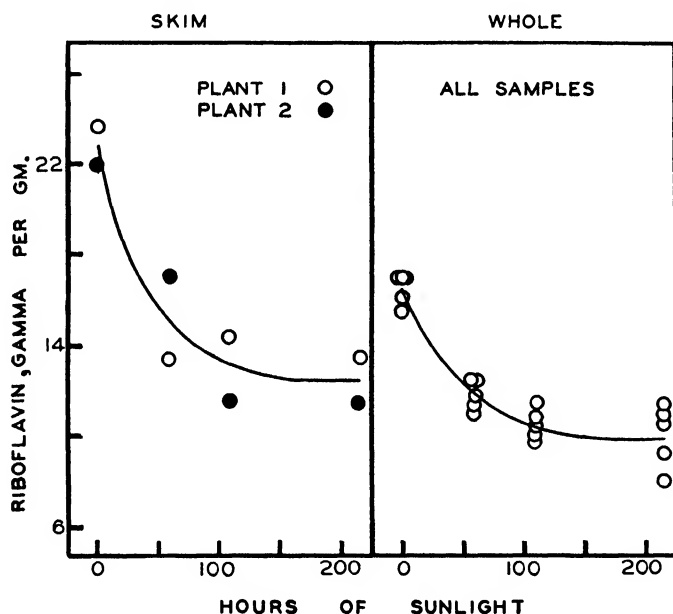


FIG. 1. The effect of sunlight on the destruction of riboflavin in milk powders from Plants 1 and 2.

hr., after which the rate of photolysis was greatly reduced. Skim-milk powders had higher riboflavin contents than whole milk powders and suffered greater destruction than the whole powders. Vitamin losses occurred at approximately equivalent rates in the skim-milk powders from the two plants, the initial differences being largely maintained throughout.

Similar mathematical assessment of the data for powders exposed to ultra-violet light showed that the only significant difference was that between skim-milk powders from the two producers. Nevertheless, there was some evidence

of riboflavin destruction (Fig. 2). The rate of destruction was about the same for both types of powders, since the curves for the skim and whole milk powders were practically parallel. The most pronounced difference in these comparisons was that resulting from exposure to the two different light sources. Sunlight caused a greater decrease in riboflavin content than ultra-violet light.

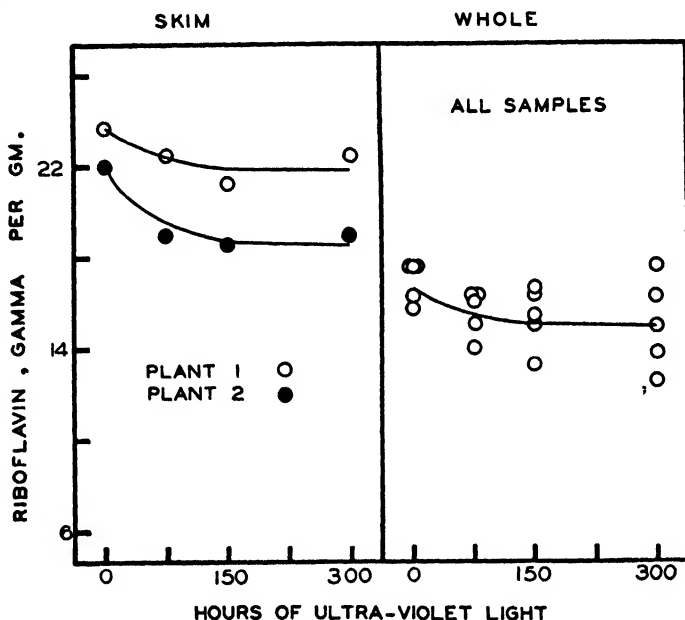


FIG. 2. The effect of ultra-violet light (3200 to 4200 Å) on the destruction of riboflavin in milk powders from Plants 1 and 2.

Variations in the intensity of the energy falling on the samples had a marked effect on the rate of photolysis of the riboflavin in skim-milk powders as is evident from the data in Table I and from a comparison of Figs. 1 and 2. In whole milk powders, the differences in energy did not greatly affect the rate of destruction of the riboflavin.

TABLE I

EFFECT OF VARIATION IN INTENSITY OF LIGHT ON DESTRUCTION OF RIBOFLAVIN;
75 HR. EXPOSURE TO A 100 WATT TUNGSTEN LAMP

Distance from lamp, ft.	Energy level, cal./sq. cm./min.	Riboflavin content (gamma/gm.)			
		Skim-milk powder		Whole milk powder	
		Initial	Final	Initial	Final
1	0.0036	22.00	12.50	17.50	10.00
4	0.0004	22.00	17.00	17.50	11.75

The data for the comparison of the destructive effect of wave bands between 4200 and 5600 Å are shown in Fig. 3. While all wave bands studied caused some destruction of the riboflavin, the greatest decrease occurred at the band having a principal wave-length of 4450 Å. From Fig. 4 it can be seen that this wave-length corresponds approximately to a maximum in the absorption spectrum of riboflavin.

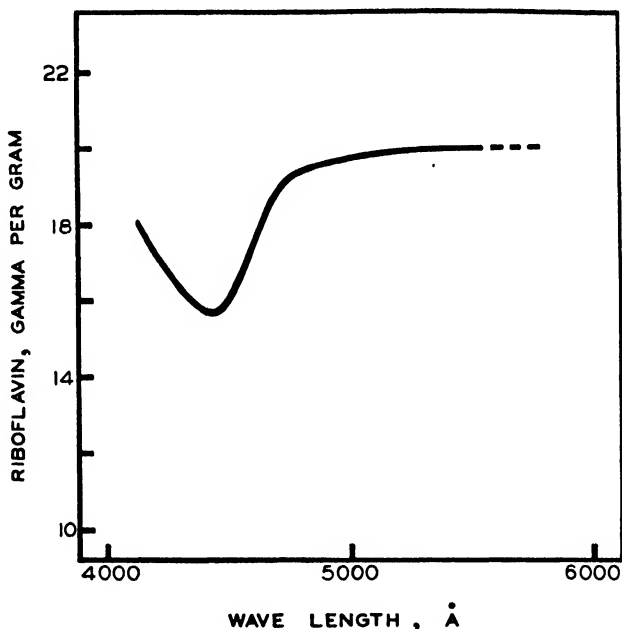


FIG. 3. *The effect of various wave-lengths of light on the destruction of riboflavin in liquid skim-milk. (Riboflavin content calculated on basis of milk solids; initial value, 22 gamma per gm.).*

Discussion

The destruction of riboflavin in the milk powders was much more rapid and complete owing to exposure to sunlight than it was as a result of exposure to ultra-violet light. This was believed to be due to the fact that the energy in the sunlight was much greater than that from the ultra-violet source, and also because the most destructive wave-lengths were not present in the ultra-violet light used in this experiment. The data in Table I showed that the greatest destruction occurred in the powders at the greatest level of light intensity. The effect was much more noticeable for skim-milk powders than it was for whole milk powders.

The wave-length of the incident light was an important factor in the photolysis of riboflavin. The marked destruction of the vitamin due to sunlight action was probably partially attributable to the presence in sunlight of the destructive wave band around 4450 Å. Further evidence of the significance of wave-length of the impinging light on the rate of riboflavin photolysis was

provided by the observation that when skim-milk powder was exposed to visible light and to ultra-violet light of equal intensity (0.0034 cal. per sq. cm. per min.) for the same length of time (75 hr.), 43% of the riboflavin was destroyed by the visible light, and only 10% was destroyed by the ultra-violet light. Only in the visible light was the destructive wave band around 4450 Å present. The fact that commercial irradiation of milk causes only a slight decrease in riboflavin content is possibly due to the absence of these destructive wave-lengths from the ultra-violet light used.

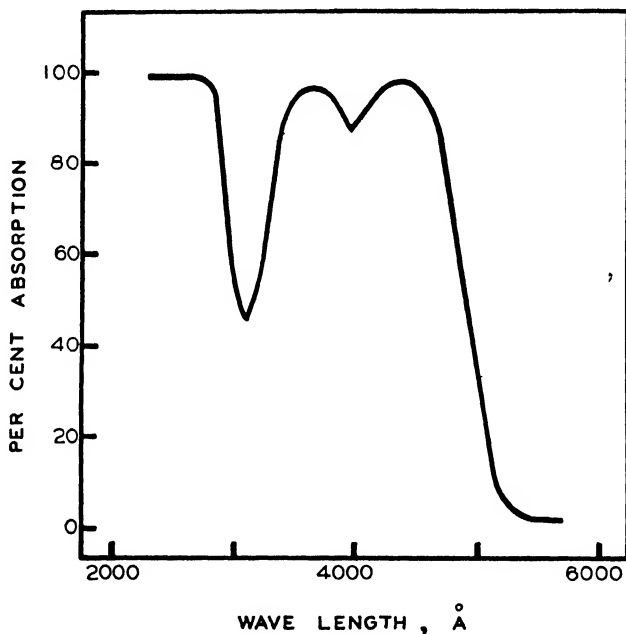


FIG 4. The absorption spectrum of riboflavin in the range 2500 to 5500 Å

The apparent decrease in the rate of destruction of riboflavin in the milk powders after prolonged exposure to sunlight has also been observed during the photolysis of alkaline solutions of pure riboflavin*. In the latter study, the results obtained from photofluorometric assays were not confirmed by microbiological determinations. Materials with chemical and fluorescent properties similar to those of riboflavin may have been measured as riboflavin in the photofluorometric analysis. Irradiation products of riboflavin have been isolated and identified (7, 8) and the fluorescent spectrum of one of these substances, lumiflavin, is similar to that of the vitamin (1, 2, 7, 13).

The decrease in rate of photolysis may also be attributed to the formation of substances that protected the vitamin from further photolysis. This problem is receiving attention in these laboratories at the present time.

* Gorham, P. Unpublished results.

Acknowledgments

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LIQUID AND FROZEN EGG

I. APPLICATION OF A FLUORESCENCE MEASUREMENT TO LIQUID AND FROZEN EGG¹

BY MARGARET REID²

Abstract

The direct measurement of fluorescence on clarified sera collected as exudate from defrosting egg distinguished between samples of frozen egg of varying quality. The method may be useful in egg drying establishments where large quantities of frozen egg are available for test purposes.

A similar serum was extracted from liquid and frozen egg by the following procedure. Approximately 50 ml. of fresh or thawed egg liquid was added to approximately 100 ml. of chloroform, mixed by gentle swirling, allowed to stand two minutes, shaken vigorously for 30 sec., and immediately centrifuged for 15 min. at 2000 r.p.m. The top layer, consisting of 15 to 20 ml. of red serum, was poured off and clarified by centrifuging for one minute, and the fluorescence of the clear serum determined. The latter measure consistently distinguished between samples of liquid and frozen egg of varying quality. The serum produced was less highly fluorescent than that obtained as exudate, and consequently the range between quality types was narrowed.

The sera extracted with chloroform from freshly broken eggs of good quality, had a higher fluorescence than that extracted from the material after standing at temperatures of 30° to 90° F. for short periods. However, the fluorescence rose when deterioration became evident. Freezing of liquid egg resulted in a decrease in fluorescent materials.

Introduction

The seasonal production of eggs, coupled with large wartime commitments, has made it necessary to store unprecedented quantities in the form of frozen blocks. No suitable test has been available for estimating the quality of liquid or frozen egg. During a preliminary investigation the following methods were tried without success: reducing sugar values measured on a tungstic acid filtrate (5, pp. 416 and 438); acid-soluble phosphorus (5, p. 461); peroxidase value (3); the fluorescence (6) of tungstic acid and trichloroacetic acid filtrates of egg melange (5, pp. 416 and 461); pH (8); fluorescence of the liquid egg, a modification of a technique applied to egg powder (4); loaf volume (7); foaming volume (7); and solids content (1). Palatability ratings on the frozen egg liquid, cooked as scrambled egg and scored by the methods used for reconstituted dried egg powder (6), were also of little value in distinguishing quality. Eggs frozen from material discarded as infertile after 7 to 10 days' incubation were frequently preferred to a Grade A product.

During the course of these investigations, it was observed that a pink watery serum separated from frozen egg while defrosting. A similar serum could be extracted from both the fresh and defrosted products by shaking with chloroform and centrifuging. Measurements of refractive index, pH,

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² Biochemist, Food Investigations.

reducing sugar, and fluorescence were made directly on this extracted serum. Of these, only fluorescence showed promise as a possible measure of quality. The present paper describes the results to date of applying the fluorescence test to sera from samples of liquid and frozen egg of varying quality.

Materials and Methods

Materials

The various qualities of liquid and frozen egg used are described in the tables.

Methods

In preliminary work, serum was obtained by collecting the exudate from thawing egg. This material was clarified by centrifuging for one minute at 2000 r.p.m., and a fluorescence measurement was made on the supernatant fluid by methods previously described (6). However, 4 to 6 lb. of frozen egg was required to produce sufficient serum, and, moreover, no serum separated from egg frozen and stored for less than a month.

Separation of a similar serum from all types of liquid and frozen egg was brought about by adding chloroform to the egg, shaking, and centrifuging. In the adapted procedure, approximately 50 ml. of fresh or thawed egg liquid, which had been well mixed and strained, was added to approximately 100 ml. of chloroform, mixed by gentle swirling to avoid emulsification, allowed to stand for two minutes, shaken vigorously for 30 sec., and immediately centrifuged for 15 min. at 2000 r.p.m. The top layer, consisting of 15 to 20 ml. of red serum, was poured off and clarified by centrifuging for one minute. The fluorescence of the clear serum was then determined.

Results

The results are given in Tables I to IV, in which the samples are listed in order of decreasing quality.

In Table I the fluorescence values of sera obtained by seepage are compared with those of sera obtained by the chloroform extraction of materials representing a wide range of quality. It will be noted that both methods showed an increase in fluorescence values as quality decreased. Fluorescence values for sera obtained by seepage were consistently higher than the values for chloroform-extracted sera. Chloroform extraction appeared to remove approximately one-third of the fluorescing materials. The higher fluorescence values for sera collected by seepage resulted in better differentiation between quality types; nevertheless the chloroform extraction procedure was used in the subsequent work reported here because of its economy of time and materials.

Table II shows the effect of storing egg liquid at temperatures ranging from 30° to 90° F. Although fluorescence of the sera increased with increasing storage temperature, storage at the lower temperatures apparently resulted in an initial decrease in fluorescing materials. This behaviour was supported

TABLE I

FLUORESCENCE OF SERA OBTAINED FROM FROZEN EGG AFTER THREE MONTHS' STORAGE
(Means of six measurements)

Description of sample	Fluorescence	
	Sera extracted with chloroform	Sera obtained by seepage
Grade A (no off-odour)	6.9	9.4
Grade C (definite off-odour)	7.6	11.9
Grade C held in shell at room temperature for three weeks prior to freezing (definite off-odour)	8.3	17.5
Incubator rejects* (strong off-odour)	21.4	33.0
Grade A broken out and held at 80° F. for 36 hr. prior to freezing (extreme off-odour)	38.8	43.7

* Infertile eggs rejected during incubation.

TABLE II

EFFECT OF TEMPERATURES ABOVE FREEZING ON
FLUORESCENCE OF SERA EXTRACTED FROM
LIQUID EGG STORED FOR 32 HR.

(Means of two measurements)

Description of sample	Fluorescence of serum
Initial, before storage	10.0
Temperature of storage, ° F.	
30*	6.0
40	7.0
50	7.5
60	8.8
70	10.0
80	13.3
90	16.5

* Held for 12 hr. only.

by a subsequent observation that freshly broken eggs of good quality had a higher fluorescence than the same material after standing at 80° F. for 16 hr. in sterile containers (see Table IV).

Similar behaviour is shown in Table III. Sera extracted from fresh Grade A eggs before freezing had a higher fluorescence value than sera similarly extracted from eggs candled as Grade A after five months commercial storage in the shell. It will be noted on examination of the standard deviations that

the variations between samples within each quality type were wide. This was not evident however where large batches of liquid egg were involved (Tables I and IV). It will also be observed in Table III that freezing consistently resulted in decreased fluorescence values.

TABLE III
MEAN FLUORESCENCE AND STANDARD DEVIATIONS FOR SERA EXTRACTED
FROM EGG BEFORE AND AFTER FREEZING

(Means of 12 measurements)

Description of sample	Fluorescence			
	Before freezing		After freezing	
	Mean	Standard deviation	Mean	Standard deviation
Grade A, one day old	12.4	1.63	7.1	0.60
Grade A, stored five months at 30° F.	10.9	1.08	10.0	1.06
Grade C, stored two months at 30° F.	14.1	2.63	10.6	1.39
Incubator rejects, candled at four and seven days, held one week at 30° F.	17.7	2.53	15.7	1.22

Table IV shows the fluorescence values for sera obtained from Grade A liquid egg, from similar material after standing, and from Grade C and lower grade materials. The fluorescence of serum extracted from freshly broken liquid egg distinguished between quality types, Grade C melange being well differentiated from Grade A. No deterioration could be detected either organoleptically or by the fluorescence measurement in liquid egg stored 16 hr. at 80° F. in sterile glass (see also Table II).

These materials were then frozen and stored in the frozen state. Directly after freezing, the range in terms of fluorescence units was greatly narrowed—only two units separated Grade A and Grade C material. After storage at 10° F., fluorescence values for Grade C and cracked eggs were lowered still further. However, this drop did not appear in the materials stored at 0° and -10° F. (the temperatures used commercially) with the result that differentiation was equivalent to that for freshly frozen material. As measured by fluorescence, only minor changes in quality occurred in frozen egg stored for six months at temperatures ranging from -10° to 10° F.

Discussion

Fluorescence measurements on the serum exuded by thawing egg are considered to have distinct promise as a quality control measure in egg drying

TABLE IV

FLUORESCENCE VALUES OF SERA EXTRACTED FROM EGG MATERIALS OF VARYING QUALITY BEFORE AND AFTER FREEZING, AND AFTER STORAGE IN THE FROZEN STATE

(Means of two measurements)

Treatment	Quality of materials used						
	Fresh Grade A* (no off-odour)	Grade A held 16 hr. at 80° F. in sterile glass prior to freezing (no off-odour)	Grade C (slight off-odour)	Cracked eggs (definite off-odour)	Musty eggs (strong off-odour)	Incubator rejects (very strong off-odour)	Mean values for treatments
Before freezing	10.0	6.3	14.0	15.0	32.0	58.2	22.6
After freezing	6.1	5.6	8.0	11.6	14.5	32.5	13.1
Three months storage at 10° F.	6.1	6.4	7.0	8.5	14.0	27.0	11.5
0° F.	6.5	7.0	8.6	8.9	14.0	26.0	11.8
-10° F.	6.7	7.2	9.4	11.8	16.4	31.0	13.8
Six months storage at 10° F.	6.3	6.5	7.0	9.7	15.9	33.0	13.1
0° F.	6.3	6.2	7.6	11.5	15.2	30.5	12.9
-10° F.	6.6	6.5	7.8	9.5	17.0	35.5	13.8
Mean values for quality types	6.8	6.5	8.7	10.8	17.4	34.2	—
Duplicate error	± 0.3			± 1.0			

* Means of six measurements.

plants. The fact that no serum separates from frozen egg stored for less than a month, and that 4 to 6 lb. is required for a measurement, make the measure unsuitable for most investigational purposes—but these restrictions do not apply to quality control in egg drying establishments, since the frozen egg is usually stored for more than a month before drying, and all the frozen material has to be thawed in any event before it can be used.

For investigational purposes, chloroform-extracted sera avoid expense in time and materials. This adapted procedure gives less critical results, but nevertheless appears to offer some discrimination between the quality of samples of both liquid and frozen egg. A more precise evaluation of the measure will be made in forthcoming papers of this series.

Acknowledgment

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SEPARATION OF STARCH AND GLUTEN

IV. APPLICATION OF A RAPID PROCESS TO FLOURS FROM VARIOUS GRADES AND TYPES OF WHEAT¹

By ¹A. L. SHEWFELT² AND G. A. ADAMS³

Abstract

A rapid process for separating starch and gluten from hard wheat patent flour (consisting of dispersal of a soft dough in water followed by screening) has been applied to pastry flour, whole wheat flour, and patent flours from the following wheats: No. 1 Northern, Hard Red Spring of high protein content; No. 2 Northern, Hard Red Spring of low protein content; No. 4 Northern, Hard Red Spring, severely damaged by frost; No. 2 C. W. Garnet; No. C. W. Amber Durum; and No. 1 Alberta Red Winter. The original separation procedure required only minor modifications in spite of varying quantities and characteristics of the glutens of these flours. Whole wheat flour required a substantially greater amount of mixing water for the preparation of a satisfactory dough. Approximately 90% of the starch present in each flour was recovered. Starches from the patent flours had protein contents ranging from 0.49 to 0.64% while that from whole wheat flour contained 1.06% of protein. Recoveries of gluten from all flours were practically complete. Crude dry gluten prepared from patent flours contained 20 to 30% of starch while the bran-gluten fraction from whole wheat flour contained 9.9%.

Introduction

Most of Canada's export and domestic trade demands a hard wheat suitable for the manufacture of high quality flour. Several types and grades of Canadian grown wheat, which fail to meet this requirement because of unsuitable gluten quality or gluten quantity, are usually available at a lower market price and merit attention as a possible source of commercial starch.

A previous communication (1) described a rapid method for separating starch and gluten from commercial hard wheat patent flour. The present study was designed to obtain information on the application of the process to flours made from various types and grades of wheat.

Materials and Methods

Representative 1 bu. lots of each of the following wheats were used as the basis for this study.*

1. No. 1 Northern, Hard Red Spring (high protein).
2. No. 2 Northern, Hard Red Spring (low protein).
3. No. 4 Northern, Hard Red Spring (frosted).
4. No. 2 C. W. Garnet.
5. No. 1 C. W. Amber Durum.
6. No. 1 Alberta Red Winter.

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² Biochemist, Industrial Utilization Investigations.

* Supplied by the Grain Research Laboratory, Board of Grain Commissioners, Winnipeg.

After samples of each of the wheats had been reserved for analytical purposes, flours were prepared from the remaining portions by means of a Buhler experimental flour mill. In addition to these prepared flours, commercial pastry and whole wheat flour were included for comparative purposes.

After trial separations on each flour, the following conditions, judged to be most generally suitable, were selected for comparative study.

Three kilograms of flour was mixed with a fixed quantity of water at 30° C. for 15 min. For the six laboratory-prepared flours, 2550 ml. was used; for the pastry flour, 2400 ml.; and for the whole wheat flour, 3300 ml. Moisture content of the various flours was sufficiently constant to render corrections for its variation unnecessary. The dough-mixing phase was followed by a 10 min. conditioning period. The remainder of the separation and also the recovery of the products were conducted as previously described (1).

Analytical methods.—Wheat samples were ground in a Wiley mill and then ball milled for six hours. These, and samples of the various flours, recovered starches, and drum-dried glutes were analysed for starch, nitrogen, and moisture. In addition, the flours were analysed for gluten content. All methods were the same as those used in the previous study (1).

Experimental Results and Discussion

The analytical data obtained for starch, protein, and moisture contents of the various wheats and their respective flours together with flour extraction values are presented in Table I. The inverse relation of starch and protein values for the various patent flours is apparent. Starch values ranged from 65.7% for the No. 1 Northern high protein wheat flour to 70.8% for the commercial pastry flour. The corresponding protein values ranged from 14.4 to 10.6%. The analytical data for the commercial whole wheat flour were of the same order as those of the representative wheat samples.

TABLE I

ANALYSES OF THE VARIOUS WHEATS AND FLOURS USED IN COMPARATIVE SEPARATIONS

Materials	%							
	Flour extrac- tion	Starch*		Protein*		Moisture		Gluten Flour
		Wheat	Flour	Wheat	Flour	Wheat	Flour	
High protein wheat	66	50.9	65.7	14.5	14.4	11.3	13.5	14.8
Low protein wheat	67	54.2	68.1	12.8	2.0	14.0	13.4	12.1
Frosted wheat	63	52.1	66.4	13.4	12.8	12.4	13.8	12.9
Garnet wheat	68	54.5	65.7	12.5	12.1	12.7	13.4	12.3
Durum wheat	51	54.5	66.1	14.2	13.1	12.7	13.6	12.5
Winter wheat	58	52.8	67.7	13.1	12.5	11.7	13.6	12.1
Pastry flour	—	—	70.8	—	10.6	—	13.5	9.3
Whole wheat flour	—	—	56.1	—	13.6	—	13.2	24.4**

* Calculated on a 13.5% moisture basis.

** Includes bran fraction.

Data on the recovery and purity of the starch and gluten fractions separated from the flours are given in Table II. Starch recovery was approximately 90% for all flours while gluten recovery was substantially complete. Crude starches from patent flours contained 0.49 to 0.64% of protein. Starch from

TABLE II.
RECOVERY AND PURITY OF STARCH AND GLUTEN FRACTIONS SEPARATED
FROM EIGHT DIFFERENT FLOURS

Type	%			
	Starch recovery	Gluten recovery	Protein content of starch*	Starch content of gluten*
High protein wheat	91	98	0.64	20.8
Low protein wheat	88	97	0.61	22.2
Frosted wheat	90	103	0.54	29.0
Garnet wheat	92	100	0.58	22.9
Durum wheat	91	98	0.54	27.1
Winter wheat	93	101	0.49	27.6
Pastry	88	99	0.51	22.9
Whole wheat	90	98	1.06	9.9

* Air-dry basis.

whole wheat flour contained 1.06% of protein. Starch contents of the crude dry glutens ranged from 20 to 30%, while that of the bran-gluten fraction from whole wheat flour was 9.9%. Thus, despite the wide range in patent flour source, the variation in amounts and purity of starch and gluten was comparatively small. Some of the variation can be explained by differences in the water-absorptive capacities of the flour, and, in practice, could be compensated for by adjusting the amount of dough-mixing water for each type of flour.

Since whole wheat flour had a higher water-absorptive capacity than the patent flours, 3300 ml. of mixing water for each 3 kgm. of flour (13.6% moisture) was required to produce a satisfactory dough. Starch obtained from whole wheat flour was somewhat dark in colour, and, while suitable as a fermentation substrate, it would require considerable refinement in order to meet commercial starch requirements. The starch content of the bran-gluten fraction was lower than that of the glutens but since this fraction represented over 25% of the original flour, the proportion of total starch which it contained was of the same order as for the other flours.

Data on the distribution of starch and protein in the separated fractions were calculated and are presented in Table III. Again the variation was comparatively small except for the proportion of total protein in the gluten fraction which varied from 76.6 to 87.6%. This variation was attributed to differences in the proportion of soluble proteins within the various flours.

TABLE II

DISTRIBUTION OF STARCH AND PROTEIN IN FRACTIONS SEPARATED FROM FLOURS

Type	Proportion of total starch, %		Proportion of total protein, %	
	Starch fraction	Gluten fraction	Starch fraction	Gluten fraction
High protein wheat	91.0	6.3	3.0	87.6
Low protein wheat	88.2	5.4	3.6	81.9
Frosted wheat	90.4	9.1	3.0	85.7
Garnet wheat	91.8	6.0	3.5	87.1
Durum wheat	91.3	5.0	2.9	82.1
Winter wheat	92.9	7.5	3.1	87.1
Pastry	87.6	4.1	3.5	76.6
Whole wheat	89.6	4.5	4.7	78.9

From the above results, it can be stated that this separation process can be applied satisfactorily to flours from many grades and types of wheat, e.g., pastry flour with a protein content as low as 10.6% was entirely satisfactory. Low protein wheat has an advantage over high protein wheat because of its higher starch content and, as a general rule, its lower market value.

The variations in gluten characteristics of the various flours had little effect on the separation. It was noted that the gluten from Durum wheat flour had a soft fluid texture and was feathery when drum-dried. With whole wheat flour separations, complete removal of the bran from the gluten was difficult and in practice the two would probably remain combined and be dried as a feed product.

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THE THIAMIN CONTENT OF MEAT¹

RITA CAMPBELL², MARY C. HILTZ³, AND ALLEN D. ROBINSON⁴

Abstract

Meats purchased in Winnipeg retail stores contained less thiamin than is reported in the literature for the same meats from other places. The values for pork compare with the ones reported in the literature, but those for other meats are distinctly lower. Arranged in descending order of thiamin content, the meats studied were: pork, variety meats, fowl, lamb, veal, and beef. Losses in cooking are quite high for fowl and some variety meats. Stewing and parboiling cause greater losses than roasting or frying.

The importance of thiamin or vitamin B₁ in our diets is too well understood to need emphasis. Among its better sources is meat—particularly pork, but also the liver, organs, and muscles of many animals. A publication of the Ministry of Food, London (10), reveals that, prior to the war, meats, as carcass weight, comprised about 12% of the food available to each Canadian consumer. By 1943 the total consumption of food per capita had increased, as had that of meat, the latter being about 13% of the total. Meat must, then, supply an appreciable part of the thiamin we consume.

There have been a number of reports published on the thiamin content of various meats. One of the most comprehensive is that of Booher, Hartzler, and Hewston (1). The following tabulation indicates some of the results reported by different workers, these being expressed on a wet basis as micrograms per gram of raw meat, except where otherwise indicated.

Meat	Hiltz <i>et al.</i> (5)	Waisman and Elvehjem (12)	McIntire <i>et al.</i> (7, 8)	Miller <i>et al.</i> (9)	Lane <i>et al.</i> (6)	Reedman and Buckley (11)	Cover <i>et al.</i> (3)	Booher <i>et al.</i> (1)
Pork loin chops	5 34 8 67	—	—	—	—	—	—	13 7 14 6
Pork loin roasts	6 10-7 78	11 1	7 4 15 2	9 2 27 8	—	—	—	—
Pork shoulder roasts	4 54-5 33	—	—	7 1 21 1	—	—	—	—
Ham	5 52 8 20	9 1	7 7 14 8	8 8 29 9	11 8	—	—	15 3 30 0
Luncheon meat	2 26-4 71	—	—	—	—	9 6 20 7*	—	—
Bacon	3 51-5 26	—	1 9	—	—	—	—	—
Chicken	—	—	—	—	—	—	—	0 8
Lamb	—	—	—	—	—	—	—	4 36
Beef	—	—	—	—	—	—	1 2 1 4	—
Veal	—	—	—	—	—	—	—	1 2- 4 3
Pork liver	—	4 4	—	1 9- 7 3	—	—	—	15
Beef liver	—	—	2 3	—	—	—	—	1 8- 3 9
Baby beef liver	—	—	1 9	—	—	—	—	—
Veal heart	—	—	7 0	—	—	—	—	—
Beef kidney	—	—	—	—	—	—	—	3 16

* Moisture-free basis.

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When meat is cooked some of the thiamin in it is destroyed and so is lost to the consumer. McIntire, Schweigert, Henderson, and Elvehjem (7) found that cooking losses for pork varied with the cooking method. Braising losses in lean meat were greater than those for roasting or broiling, though the total retention of thiamin in meat and drippings was the same, irrespective of the cooking method. Waisman and Elvehjem (12) report losses as great as 50, 55, and 56% in roasting pork, veal, and beef, respectively. That frying losses were less, they suggest, may be due to the shorter cooking time required. Stewed kidney and heart of beef showed losses of 40 and 57%. Hiltz, Robinson, and Levinson (5) found cooking losses for pork to vary from 7 to over 80%. Roasting caused losses of approximately 20% in shoulder butt roasts and 40% in ham. Frying pork chops caused losses up to 38%. Extremely high losses occurred when bacon was fried. Cover, McLaren, and Pearson (3) found thiamin retention in rare and well done rib roasts of beef to be 75 and 69%, respectively.

It was decided to investigate further the amounts of thiamin in meat by analysing meat purchased in Winnipeg retail stores. This would provide additional data to that available in the literature. It would indicate, too, whether the amounts of thiamin in meat sold in this locality are the same as those in meat sold elsewhere. Differences might be expected, since animals in different areas are often provided with different kinds of feed. It was decided, too, to investigate cooking losses of thiamin for the meat studied.

Experimental

The meats used in this investigation were beef, veal, lamb, pork, variety meats, and poultry. Standard household cuts were purchased for uncooked analysis. Similar adjacent cuts were purchased for cooking studies. Fowl, pork and beef liver, and lamb and beef kidneys were divided in halves and a half of each assayed uncooked and a half used for cooking studies. Separate baby beef hearts were used for assays of uncooked and cooked meats. The white and dark meat of fowl were analysed separately.

Each cooked cut was weighed and divided into meat, bone, visible fat, and drippings, which were weighed separately. The meat alone was retained for analysis, since in previous investigations reported by Hiltz, Robinson, and Levinson (5) it had been found that the amount of thiamin in dripping is negligible. The meat was ground in a meat grinder to make it homogeneous. Samples were taken for duplicate thiamin assays and for duplicate moisture tests. In the analysis of raw samples, meat was removed from the bone and weighed, as were the bone and visible fat portions. The meat was put through a meat chopper twice. Duplicate samples were taken for thiamin and moisture assays.

Meats were cooked following standard methods of cookery for general household usage as outlined by the Committee on Preparation Factors, National Cooperative Meat Investigators (2). Oven temperatures, internal temperatures—as shown by a Taylor meat thermometer at the centre of the

roast—and cooking times were noted. The cooking of roast meats was done at specified oven temperatures until the required internal temperature was reached. Where the use of a meat thermometer was not feasible, meats were cooked a recommended time as specified in the above reference, the time being varied according to the weight of the cut. Beef steaks and veal cutlets, pork and lamb chops were pan broiled in a preheated skillet to a well done stage. Stewing beef from the neck was simmered in water to cover until tender. The cooking of variety meats is described in Table V. A 5 gm. portion of lard was used in broiling pork liver. Lamb and beef kidney were soaked in cold water for one hour before parboiling.

Thiamin was determined by a modification of the Hennessy and Cerecedo (4) method used in a previous investigation by Hiltz, Robinson, and Levinson (5). The method was checked with two outside standards, commercial Winthrop's Vitamin B₁ Standard, and a thiamin solution of known strength provided by Dr. Hoffer of Western Canada Flour Mills Co., Ltd., of Winnipeg. Moisture was determined by the vacuum-oven method.

The experimental data are presented in Tables I to V, inclusive. These show thiamin content and cooking losses for beef and veal, pork, lamb, fowl, and variety meats. Each figure represents the average of duplicate determinations made on the edible portion. The values are higher than if they

TABLE I
THIAMIN CONTENT OF RAW AND COOKED BEEF AND VEAL

Meat	Thiamin (wet basis), μgm./gm.		Cooking treatment			Thiamin lost in cooking, %
	Uncooked	Cooked	Internal temp., ° F.	Oven temp., ° F.	Cooking time, min.	
Rump roast	0.20	0.30	165	315-25	129	—
Prime rib roast	0.24	0.29	165	315-25	102	10.2
Round bone shoulder roast	0.19	0.23	165	315-25	94	5.5
Porterhouse steak	0.21	0.30	—	—	12	1.0
Blade roast	0.20	0.29	165	315-25	102	1.3
Sirloin steak	0.30	0.39	—	—	18	13.1
Wing roast	0.21	0.32	165	315-25	116	—
Round steak	0.28	0.33	—	—	19	19.9
Neck stewing meat	0.44	0.40	—	—	92	74.8
Veal cutlets	0.51	0.27	—	—	14	67.1
Veal round steak	0.50	0.82	170	310-20	124	—

had been based on the inedible part of the meat as well. Because the thiamin contents are small they are expressed to hundredths of a microgram, rather than to tenths as is usually done. Percentage loss was calculated from thiamin contents reduced to a dry basis. Essential cooking data—time, oven temperature, and internal temperature—are included in the tables.

TABLE II
THIAMIN CONTENT OF RAW AND COOKED PORK

Meat	Thiamin (wet basis), $\mu\text{gm.}/\text{gm.}$		Cooking time, min.	Thiamin lost in cooking, %
	Uncooked	Cooked		
Loin roast No. 1	6.15	6.46	85	18.4
Loin roast No. 2	7.79	8.36	108	23.3
Chop No. 1	9.64	7.16	10	58.5
Chop No. 2	8.10	9.01	9	22.1
Chop No. 3	7.33	8.69	7	11.1
Chop No. 4	7.52	9.63	7	17.0
Chop No. 5	10.04	12.32	9	24.0

Roasts were cooked to an internal temperature of 182° F. in an oven whose temperature ranged from 310 to 350° F.

TABLE III
THIAMIN CONTENT OF RAW AND COOKED LAMB

Meat	Thiamin (wet basis), $\mu\text{gm.}/\text{gm.}$		Cooking time, min.	Thiamin lost in cooking, %
	Uncooked	Cooked		
Hind leg lower portion	0.50	0.57	113	22.4
Hind leg upper shank	0.36	0.42	130	11.7
Neck roast	0.18	0.19	127	31.5
Breast and shank	0.21	0.37	112	—
First rib chop	0.34	0.44	11	—
Last rib chop	0.36	0.44	10	10.4
Loin chop No. 1	0.63	0.74	10	15.8
Loin chop No. 2	1.18	1.30	12	23.4

Roasts were cooked to an internal temperature of 175° F. in an oven whose temperature ranged from 310 to 320° F.

Discussion

The values obtained for the thiamin content of beef vary from 0.19 to 0.44 $\mu\text{gm.}$ per gm. of raw meat. This is somewhat lower than those reported by Cover *et al.* (3), which are from 1.2 to 1.4. Cooking losses are not very large except for stewing beef. Here a large part of the thiamin may have been extracted by the cooking water. Other beef cuts show losses up to 20%. The amounts of thiamin found in veal, 0.50 and 0.51 $\mu\text{gm.}$ per gm., are lower than those reported by Booher, Hartzler, and Hewston (1). The data on cooking losses are not sufficient to justify drawing conclusions at this time. The two cuts of veal examined contained more thiamin than beef but could not be considered good sources of the vitamin.

The thiamin content of pork roasts as shown in Table II is comparable with the values reported in the literature. The values for chops are of the same order as those for roasts. Cooking losses for roasts are 18.4 and 23.3%, while for chops they are from 11.1 to 24.0%, with one chop showing a loss of 58.5%.

TABLE IV

THIAMIN CONTENT OF RAW AND COOKED FOWL

Meat	Thiamin (wet basis), $\mu\text{gm./gm.}$		Cooking treatment		Thiamin lost in cooking, %
	Uncooked	Cooked	Oven temp., ° F.	Cooking time, min.	
<i>Chicken broiler</i>			380	22	—
Light meat	0.26	0.36			—
Dark meat	0.49	0.47			78.1
Liver	2.02	—			—
<i>Roast chicken</i>			325-50	80	—
Light meat	0.44	0.36			40.3
Dark meat	0.63	0.46			49.3
Liver	—	2.03			—
<i>Stewing fowl</i>			—	155	—
Light meat	0.21	0.06			78.0
Dark meat	0.27	0.11			74.1
Cooking liquid	—	0.03			—
<i>Roast turkey</i>			350	105	—
Light meat	0.27	0.25			26.2
Dark meat	0.38	0.36			37.1
Liver	—	1.06			—

TABLE V

THIAMIN CONTENT OF RAW AND COOKED VARIETY MEATS

Meat	Thiamin (wet basis), $\mu\text{gm./gm.}$		Cooking treatment	Thiamin lost in cooking, %
	Uncooked	Cooked		
Pork liver	1.19	1.32	Broiled 20 min.	21.4
Beef liver	1.05	1.24	Parboiled 15 min.	14.5
Lamb kidney	1.49	1.87	Parboiled 10 min.	52.1
Beef kidney	1.98	2.08	Parboiled 10 min.	46.0
Baby beef hearts	1.90	1.61	Baked 80 min.	52.0
	—	1.87	Boiled 105 min.	63.0

Thiamin values for cuts of lamb assayed are presented in Table III. They are from 0.18 to 1.18 $\mu\text{gm. per gm.}$ These are lower than those reported by Booher *et al.* (1). Cooking losses for roasts and chops varied from 10.4 to 31.5%.

The thiamin content of fowl as shown in Table IV is less than that reported by Booher *et al.* The amount of thiamin in dark meat is higher than in white. This has been reported by Waisman and Elvehjem (12). Cooking losses are quite high, especially in stewing fowl. The data show that a part of this thiamin was not destroyed but was extracted by the cooking liquid.

The variety meats listed in Table V contain appreciable amounts of thiamin, but less than those reported by McIntire, Schweigert, Herbst, and Elvehjem (8). Cooking losses for livers were 14.5 and 21.4%. Those for kidneys and baby beef hearts were quite high, being from 46.0 to 63.0%.

While the number of samples tested was not sufficient to permit one to draw hard and fast conclusions, the results do indicate that, with the possible exception of pork, meats sold in Winnipeg retail stores contain less thiamin than has been reported for meat elsewhere. The order of decreasing thiamin contents is: pork, variety meats, fowl, lamb, veal, and beef. This is the same order as results reported elsewhere, save for fowl which other investigations place below beef.

Cooking losses for most meats are less than 25%. Exceptions noted include fowl and variety meats. A comparison of cooking temperatures and times with losses reveals no apparent relation to those factors. A more important cause of loss seems to be the use of water in the cooking process, as in the stewing of beef and fowl and the boiling and parboiling of variety meats. This extracts the thiamin from the meat. It is not destroyed completely, but is available in part in the cooking water.

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RESIN-RUBBER FROM CANADIAN GROWN PLANTS

V. METHODS OF EXTRACTION FROM POD HULLS OF THE COMMON MILKWEED¹

BY R. V. TOMKINS² AND N. H. GRACE³

Abstract

Yields of 2 to 3% of a resin-rubber gum from milkweed pod hulls were obtained by a process that included alkaline digestion and pebble milling. The effects of various digestive treatments and milling procedures in laboratory and pilot plant are reported. The pod hulls contained 10% of resin-rubber, but 40% of this was lost during digestion. However, the resin-rubber content of the digested material was 17%. The pebble mill comminuted the plant tissue and agglomerated the resin-rubber, the milling conditions having a marked effect on the efficiency of this operation.

Introduction

Seed pod hulls of the common milkweed, *Asclepias syriaca* L., are available from the mechanical separation of floss that is being produced in large quantities as a substitute for kapok. Since the unopened pods contain approximately equal amounts of floss, seeds, and hulls, the available quantity of hulls equals the weight of floss that is produced. Consequently, a substantial bulk of hulls is available and the amount will probably increase if the floss industry is maintained in the post-war period. Economic utilization of the pod hulls would assist this new industry to meet the inevitable competition from kapok.

Consideration has recently been given to milkweed as a source of a mechanically extracted resin-rubber gum (2). This extraction process has been carried to pilot plant scale using milkweed leaves as raw material (3). The extraction of resin-rubber from pod hulls on a laboratory scale has also been reported (2). This communication describes both laboratory and pilot plant studies of the processes involved in the extraction of resin-rubber from the pod hulls.

Materials and General Methods

Materials and Equipment

Pod hulls obtained from Petosky*, Michigan, served as the raw material for the extraction reported herein. Eight samples, collected at random, were analysed. The following average values were obtained: moisture 8.6%, resin-rubber 9.85%, and residue 81.55%. These analytical values were

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* Baled pod hulls were kindly supplied by the Hemp Division, Commodity Credit Corporation, Petosky, Mich. These hulls from the mechanical extraction of floss contained approximately 10% of floss and seeds.

used for all calculations except in the study of digestion conditions in the laboratory.

The alkali used throughout this investigation was commercial flake sodium hydroxide.

Laboratory and pilot plant studies utilized steam-heated digestion equipment, cheesecloth filters, porcelain-lined pebble mills, and vibrating stainless steel screens, which have been described previously (2, 3). The laboratory mill had a gross capacity of 55 litres and an internal diameter of 16 in. The pebble charge occupied 25% of the gross volume and the slurry charge, 33%, leaving 42% free space. Flint pebbles, average diameter 1.1 in., were used. The larger scale pilot plant mill had an internal diameter of 33 in. and a gross capacity of 140 gal. The pebble and slurry charges were proportioned as in the small mill, but larger pebbles, average diameter 2.3 in., were used. From information on the grinding of minerals, 80% of theoretical critical speed was selected for maximum capacity (1, pp. 29-31). Rotation of the laboratory mill at 53 r.p.m. attained this value, but the larger pilot plant mill was held to 70% (32 r.p.m.) to avoid excessive wear on the porcelain lining.

Procedure

The general method involved digestion, washing to the required pH on the filter, pebble milling followed by screening, and return of the oversize to the mill for final agglomeration.

Analysis of Pod Hulls and Resin-rubber Gums

The analytical procedures have been described (4). The rubber content of the mechanically extracted resin-rubber is low, and error in its determination results if the acetone extraction is not complete. However, the total resin and rubber obtained by successive 24-hr. extractions with acetone and benzene is relatively constant, and this value, designated "resin-rubber," is used throughout this communication. The remainder, insoluble in acetone and benzene, is termed "residue." Percentage yields are reported on a basis of dry resin-rubber, and air-dry pod hulls.

Experimental

In a preliminary series of laboratory extractions of pod hulls, alkali concentration, temperature, and time of digestion were varied but milling conditions were maintained constant. The results showed great irregularity in yield from duplicates. No resin-rubber was agglomerated from material digested in water or 0.5% alkali. Subsequently, laboratory studies were designed to elucidate sources of gross variability and to obtain data useful in scaling up the process to the pilot plant.

Application of laboratory procedures, with only minor modifications, to pilot plant scale extractions resulted in successful agglomeration of the resin-rubber. Pilot plant experiments were performed to gather information on digestion and milling conditions from which economically optimum procedures might be selected.

Digestion

Alkaline digestion was used as a means of releasing resin-rubber from the plant tissue and to effect concentration of the resin-rubber by removing part of the residual material. As this treatment also removed some resin-rubber, the effects of alkali concentration, temperature, and duration of digestion were investigated to determine which conditions led to maximum concentration of resin-rubber with minimum loss of this desired fraction.

A 7 kgm. mass of pod hulls was mixed, sampled, and analysed. The material contained 6.2% of moisture, 9.6% of resin-rubber, and 84.2% residue. (The resin-rubber content on a moisture-free basis was 10.3%.) Batches of hulls (250 gm.) were digested, the ratio of solution to pod hulls being maintained at 15 : 1. From previous work (2), basic digestion conditions of two hours at 121° C. in 1.5% alkali were selected. The three factors were varied individually around this basis over the ranges indicated in Figs. 1 and 2. In addition, the effect of a 15 min. water extraction at 100° C. prior to digestion was investigated. The duration of the treatment was measured from the time the charge reached the desired temperature. The digested hulls were washed to pH 9.5 and moistures determined on the total drained mass to avoid sampling errors. Analysis of the dried material permitted calculation of loss of resin-rubber and residue resulting from various digestive treatments.

Eight 1-kgm. samples of pods were taken at random, analysed, and digested. The resin-rubber contents before and after digestion are compared in Table I. It is apparent that the precision of results after digestion is comparable to the variability of the source material.

TABLE I
VARIANCE IN REPLICATE SAMPLES BEFORE AND AFTER DIGESTION
Digestion conditions: two hours at 100° C. in 1.5% alkali

	Before digestion		After digestion	
	Resin-rubber	Residue	Resin-rubber	Residue
Gm. per kgm. air-dry hulls	98.5	815.5	59.4	306.0
Standard deviation, gm.	7.4	15.8	4.46	8.9
Coefficient of variability, %	7.50	1.94	7.51	2.91

The reduction of the residue content of the pod hulls during digestion and the accompanying loss of resin-rubber are shown in Fig. 1. Digestions at lower alkalinity and temperatures, and for shorter times, resulted in relatively greater loss of residue than of resin-rubber. Loss of resin-rubber increased as the treatment became more drastic, until finally a greater percentage of this fraction was lost. Water digestion before the alkali treatment had little effect. The flatness of the curves describing temperature and time

effects is probably due to the alkali concentration (1.5%) at which these trials were performed. The losses were not entirely due to chemical action, as small particles were lost during filtration.

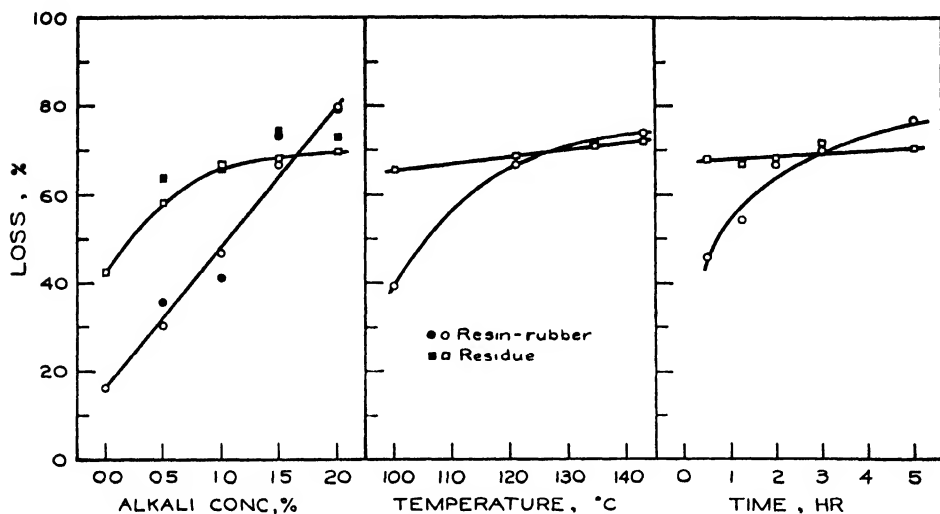


FIG. 1. Effect of digestion conditions on loss of resin-rubber and residue. Solid points indicate prior water extraction.

Residual resin-rubber in the digested pod hulls is shown in Fig. 2. As the intensity of treatment increased, the resin-rubber content of the digested material rose to a maximum, then fell below that of the undigested hulls

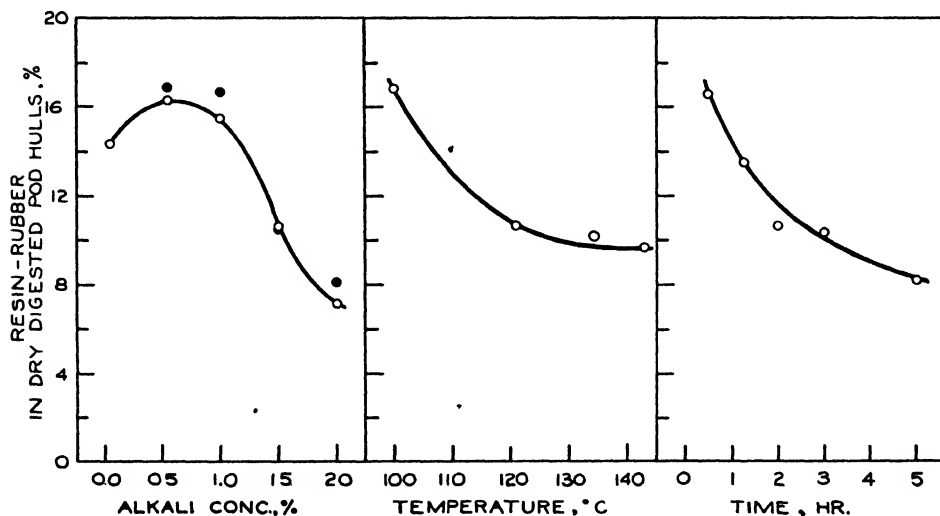


FIG. 2. Effect of digestion conditions on concentration of resin-rubber after digestion. Solid points indicate prior water extraction.

(10.3%). Over the range investigated, alkali concentration had most marked effect, and the use of lower temperatures and a short cooking time is indicated.

The dependent variables are plotted in Figs. 3 and 4 to show the continuity of effects of cooking treatments. Fig. 3 indicates that the loss of residue approaches a limiting value at about 72%, whereas resin-rubber loss shows no such limit. The curve in Fig. 4 shows a pronounced maximum at 17% of resin-rubber in the digested material. Obviously, digestion conditions in the vicinity of the maximum are most desirable, as more drastic treatment resulted not only in further loss of the desired fraction but also in failure to

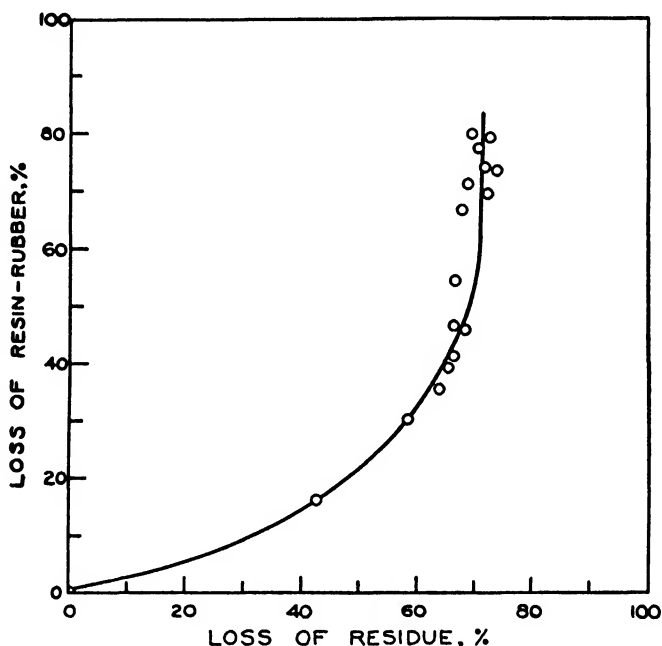


FIG. 3. Relation between loss of resin-rubber and loss of residue during digestion.

increase the resin-rubber content of the digested hulls. The points near the maximum correspond to the following digestion conditions: 2 hr. at 121° C. in 0.5 and 1.0% alkali; 2 hr. at 100° C. in 1.5% alkali; and 0.5 hr. at 121° C. in 1.5% alkali. Material digested in 0.5% alkali yielded no resin-rubber when milled. Although the resin-rubber content of the mill charge was high, apparently digestion did not release the resin-rubber from the plant tissue sufficiently for successful agglomeration. The remaining three sets of conditions were selected, therefore, for pilot plant trials.

Duplicate millings of hulls digested as indicated above were performed in the pilot plant. The cooked material was washed to pH 9.5, milled at 3.6% solids for three hours, screened, and the oversize remilled with wash water for 1.5 and 0.5 hr. The yields are compared in Table II. Hulls digested

in 1.0% alkali did not mill as well as those obtained by the other digestive treatments. Subsequent milling experiments made use of pod hulls digested for two hours at 100° C. in 1.5% alkali.

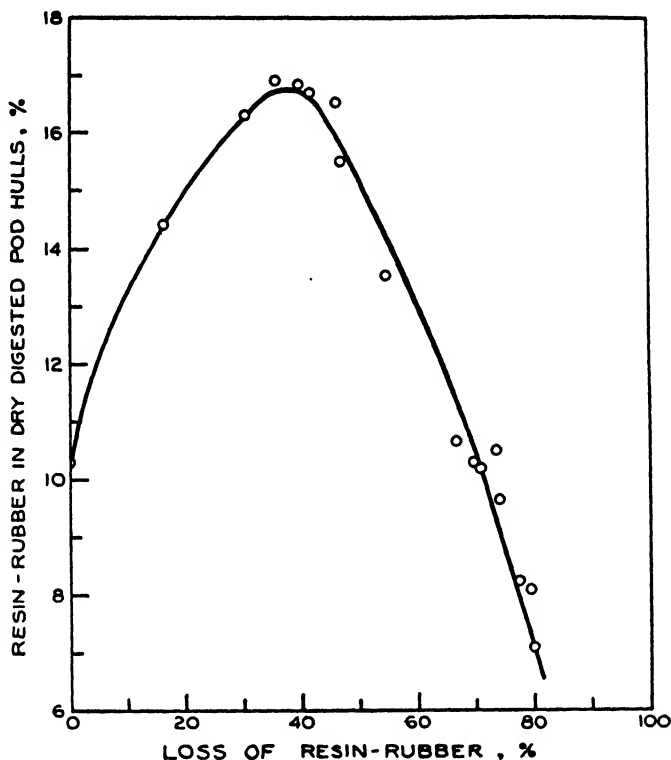


FIG. 4. Relation between concentration of resin-rubber after digestion and loss of resin-rubber during digestion.

TABLE II

YIELDS OF RESIN-RUBBER AFTER VARIOUS DIGESTIVE TREATMENTS

Duration, hr.	Digestion		Yield of resin-rubber, %
	Temperature, ° C.	Alkali conc., %	
2	121	1.0	1.1
2	100	1.5	1.7
0.5	121	1.5	1.7

Washing

Removal of dissolved substances and excess alkali was accomplished by draining and washing the digested pods with warm water. Two-kilogram samples of pod hulls were digested at 100° C. for two hours in 1.5% alkali and washed to different pH values. Subsequently, duplicate millings of four

hours at 1.8% solids and loading temperature of 45° C. were performed, and the yields of resin-rubber recorded. Sulphuric acid was added at a late stage of washing to obtain material with pH less than 7.

The data in Table III indicate a maximum yield as the pH approaches 7, with low yield from both strongly acid or alkaline material. The low yields were probably due to the reaction of residual alkali or acid with the resin

TABLE III
EFFECT OF PH ON YIELD OF RESIN-RUBBER

pH of digested, washed sludge	Yield of resin-rubber, %	pH of digested, washed sludge	Yield of resin-rubber, %
11.8	0.3	7.6	3.1
10.1	2.2	6.3*	1.5
9.5	2.4	4.5*	1.1
8.3	2.5	3.0*	0.8

* Acid added after washing.

fraction during milling. A pH of about 9 was readily attained with little water and labour, but greatly increasing amounts of both were required to reduce this appreciably. More thorough washing would have to be justified by a balance of additional yield against added cost.

Pebble Milling and Screening

The digested, washed pod hulls were pebble milled and the agglomerated resin-rubber concentrated by screening. Gelatinization of the cellulose and emulsification of the resin-rubber must be minimized if successful separation of the desired fraction is to be attained. Since the effectiveness of the milling procedure was judged by the amount retained on the screen, the importance of the screening operation is obvious. Indeed, the wide variation in yield for duplicates in preliminary work was traced to the use of a 12 mesh screen, which allowed varying amounts of resin-rubber to pass. A microscopic study of the particles passing the 12 mesh screen indicated that 80% of the resin-rubber would be retained on 60 mesh. The adoption of this screen eliminated the variation mentioned, but required the use of an additional 30 min. washing period to agglomerate the resin-rubber, as a greater portion of the residue was also retained. The effects of slurry consistency and temperature on the yield and the effects of duration of milling and screen size on the efficiency of the screening operation were investigated in the laboratory.

The percentage of resin-rubber and residue retained on 20, 60, and 100 mesh screens after three, four, and five hours' milling is shown graphically in Fig. 5. The use of 100 mesh slightly increases resin-rubber retained but is offset by excessive amounts of residue and the extra time required for

screening. A 20 mesh screen is effective for separation of the fractions but retains only slightly more than half the resin-rubber present in the slurry. Screening on 60 or 100 mesh after three hours' milling does not effectively separate the two components. Extending the milling period to

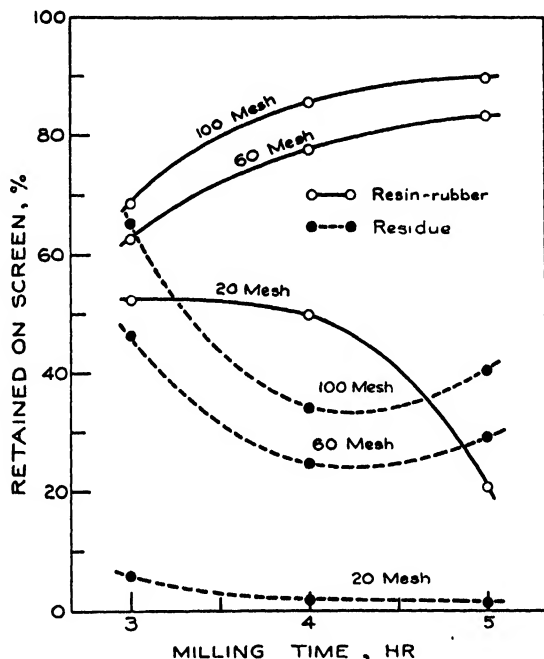


FIG. 5. Separation of resin-rubber and residue by 20, 60, and 100 mesh screens after milling three, four, and five hours.

five hours results in a noticeable gelatinization or swelling of the residue, causing more of this undesirable material to be retained than after four hours' milling. The separation at four hours on 60 mesh appears most satisfactory.

The effects of slurry consistency and slurry temperature are shown in Table IV. At the higher consistencies, the slurry became excessively thick and difficult to handle, with reduced yields. Solid contents lower than 2.4% not only reduced the capacity of the mill (i.e., less resin-rubber per charge) but the percentage yield fell off. The slurry temperature had little effect on the yield except at 5° C.

The change in grinding characteristics accompanying increase of mill and pebble size necessitated a restudy of conditions of slurry consistency and duration of milling. Consideration of the heavier stones and longer falls in the large mill suggested the possibility of successful agglomeration using thicker slurries and shorter milling times than were required under laboratory conditions.

TABLE IV

EFFECT OF SLURRY CONSISTENCY AND SLURRY TEMPERATURE ON
YIELD OF RESIN-RUBBER (LABORATORY)

Milled four hours, screened on 60 mesh

Slurry temp., loading, ° C.	Slurry consistency, % solids	Yield resin-rubber, %
45	1.2	1.1
45	1.8	2.4
45	2.4	3.0
45	3.0	0.9
5	1.8	1.6
25	1.8	2.5
45	1.8	2.4
65	1.8	2.5

The resin-rubber from preliminary pilot plant millings was excessively sticky and difficult to handle, but charging the mill with cold water (5 to 10° C.) eliminated this trouble. After the main milling period the ground hulls were passed over a 60 mesh vibrating screen and the oversize returned to the pebble mill with fresh water and milled an additional one and one-half hours. The resin-rubber particles were agglomerated to pieces about 1 in. in diameter by this time. The charge was drained through a coarse screen to retain the pieces of resin-rubber, and the oversize was again returned to the mill with water for a half-hour final agglomeration period. At the end of this period, the resin-rubber was removed as one or two large pieces.

The yields of resin-rubber obtained under various milling conditions are given in Table V, as pounds recovered per milling, percentage yield, and time-rate of yield. In batch milling the yield per milling is of great importance, but, were the process to be made continuous, the time rate of yield would govern conditions to be selected. A pronounced maximum is attained with four hours' milling at 3.6% solids, but it is possible that a maximum for each slurry consistency would occur at a different time. However, above 3.6% solids, the slurry becomes excessively viscous, entailing screening difficulties, while below this value the capacity of the mill per charge decreases in a batch process. Extending the period of milling beyond five hours, as previously mentioned, results in gelatinization or swelling of the cellulose, which hinders agglomeration.

The crude resin-rubber removed from the pilot plant mill was remarkably consistent as to proximate composition, averaging 40% of water, 5% of residue (largely silica from the mill lining), and 55% of resin-rubber. The chemical nature of the resin fraction has been reported (5). The resin-rubber may be stored for short periods with little decomposition under flowing water changing every four or five hours.

The yields obtained in the pilot plant were not as high as in the laboratory, probably owing to the more violent action in the pebble mill causing a greater

TABLE V

EFFECT OF SLURRY CONSISTENCY AND DURATION OF MILLING ON YIELD OF RESIN-RUBBER (PILOT PLANT)

All charges digested two hours at 100° C., 1.5% alkali

Milling time, hr.	Slurry consistency, % solids	Resin-rubber charged to mill*, lb.	Resin-rubber recovered, lb.	Over-all yield resin-rubber, %	Yield rate, lb./hr. milling**
4	1.8	1.18	0.21	1.1	0.03
4	2.7	1.77	0.36	1.2	0.06
4	3.6	2.36	0.94	2.3	0.16
4	5.4	3.54	0.78	1.3	0.13
2	3.6	2.36	0.52	1.3	0.13
3	3.6	2.36	0.69	1.7	0.14
4	3.6	2.36	0.94	2.3	0.16
5	3.6	2.36	0.53	1.3	0.08
6	3.6	2.36	0.00	0.0	0.00

* Based on analysis of digested hulls.

** Milling time plus 2 hours' washing.

amount of emulsification of the resin-rubber. The milling operation is extremely complex, and caution should be observed in interpreting the data, as they are valid only under carefully defined conditions and with specific equipment.

Acknowledgment

The writers' thanks are due to Dr. R. W. Watson of the National Research Laboratories and his staff for their excellent analytical work, and to Mr. G. D. Powers, Laboratory Assistant, National Research Laboratories, for technical assistance in conducting a number of experiments.

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FURTHER STUDY OF THE USEFULNESS OF DIFFERENT TYPES OF SHORTENING WHEN INCORPORATED IN BISCUITS AT VARIOUS LEVELS AND WITH DIFFERENT BAKING TEMPERATURES¹

BY E. W. CRAMPTON² AND MARGARET FRANCES MILLS³

Abstract

One hundred and ninety-two 30-day-old male white rats were fed diets containing four different types of shortening (compound animal-vegetable, blended vegetable, hydrogenated vegetable, and lard) incorporated at 0, 8, 16, and 24% levels by weight and baked at 375° and 425° F. Diets were mixtures of flour, milk powder, shortening, salt, and bone meal with supplementary allowances of vitamins A, D, and B₁. The proportion of ingredients was adjusted to maintain protein at 16% by weight. The relative nutritive value of the diets was measured by growth of rats, digestibility of the diet, and the proportion of fat deposited in the livers and carcasses.

Gains decreased with increasing fat level, apparently owing to a reduction of the proportion of protein to non-protein calories from the replacement of carbohydrate by fat.

Digestibility of the fat component was unaffected by baking temperatures or level of fat in the ration. Lard was slightly more digestible than the other types which included vegetable fats. Rats fed diets baked at 425° F. made slower gains than those on the diets baked at 375° F. This was not traceable primarily to heat damage to the fat but more probably to some effect on the protein fraction.

Introduction

Owing to the influx of a variety of commercial shortenings replacing lard and butter for cooking purposes, the comparative nutritive value of these fats and of the products into which they are baked for consumption has become of renewed interest to nutritionists.

One of the factors affecting the results obtained with fat-containing diets is the amount of fat in the diet. Both Osborne and Mendel (17) and Smith and Carey (21) obtained considerably better growth in rats on fat-free diets than on combinations in which fat was included. On the other hand, Frank (5) found more rapid gains on diets containing 80% fat and 15% protein, than on "normal" rations. Osborne and Mendel (18) were unable to substantiate Frank's findings. They found that the high fat rations caused failure of appetite. This was perhaps traceable to imbalance in the diets with

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respect to essential components. Later studies (Levine and Smith (11), Hoagland and Snider (7), McAmis, Anderson, and Mendel (12)) have indicated that fat as such is not the cause of unsatisfactory nutritive values in rat diets.

The specific fat employed has not been found to be of marked importance. Most of the commonly used shortenings have shown apparent digestibility of about 95% (Holmes and Deuel (10)), which means that for practical purposes there is little difference between them in so far as energy value is concerned (Hoagland and Snider (8), Deuel, Movitt, Hallman, and Mattson (4), Deuel and Movitt (3), and Hoagland and Snider (9)).

Heating of the fats, as in baking, however, has been shown by Morris, Larsen, and Lippencott (16) to damage their feeding value for rats, while Roffo (19, 20) reports that diets containing heated fats may cause tumours in the stomach.

In a preliminary study of the nutritive value of shortenings (Crampton and Mills (2)), it was found that increasing the percentage fat of the diet at the expense of carbohydrate, on a weight basis, resulted in a sharp decline in feeding value as measured by growth of rats. The effect of a baking temperature of 425° F. for 15 min. on the complete diet was also detrimental, though this might be traceable to damage to proteins rather than to the fat fraction. These results seemed of sufficient importance that the hereinafter described test was carried out to obtain further data on this problem.

Experimental

The general plan of this test was to feed to young growing rats rations in which the four shortenings to be tested were incorporated at four different levels and the diets baked at two different temperatures.

The relative nutritive values of the final diets as fed and of the fat fractions were measured by the growth of the rats during the test period and the digestibility of the diet and diet fractions. Measurement of the fat deposition in the bodies and livers of the rats was made in the hope of obtaining further information relative to the nutritive properties of the diets.

The feeding trial involved three replicate 28-day tests, each of 64, 25- to 30-day-old, male white rats. The rats were allotted at random to individual wire-bottomed cages, where the diets and water were provided *ad libitum*. Supplements of vitamins A and D and thiamin (0.8 mgm. per week) were administered orally twice weekly.

The rations fed consisted of ingredients that could be made into edible biscuits. The percentage composition of the four different mixtures is shown in Table I.

The milk powder was increased and the flour decreased as the fat was increased in these mixtures in order to maintain a constant protein level throughout.

TABLE I
DESCRIPTION OF MIXTURES USED

Ingredient or fraction	Fat level			
	0%	8%	16%	24%
Formula				
Flour	81.5	69.5	58.5	46.5
Milk powder	16.0	20.0	23.0	27.0
Fat	0.0	8.0	16.0	24.0
Bone meal	2.0	2.0	2.0	2.0
Salt	0.5	0.5	0.5	0.5
Analysis (dry matter)				
Protein	16.0	15.8	15.4	15.6
Fat	0.0	8.6	16.6	24.6
Carbohydrate	80.2	71.6	63.7	55.6
Ash	3.8	4.0	4.3	4.2

At each fat level, four different shortenings were employed as follows:—

1. Compound animal-vegetable fat—commercial Jewel shortening, manufactured by Swift Canadian Company—48% animal fat and 52% vegetable oils. Melting point, 47.5° C.
2. All hydrogenated vegetable fat—commercial Covo S.S., manufactured by Lever Brothers Limited from peanut oil. Iodine value, 58; melting point, 42° C.
3. Blended vegetable fat—prepared in Macdonald College Chemistry Department—10% stearin and 90% soybean oil. Melting point, 47° C.
4. Pure animal fat—commercial lard.

The diets were mixed and baked into commercial biscuits at two different baking temperatures, 375° and 425° F., by Harrison Brothers Limited—the Pom Bakers, Montreal. Subsequently the biscuits were oven-dried below 100° C. and ground for feeding.

A factorial type of design was used as indicated in Appendix Table II. This design made possible a partition of the variance into that traceable to replicate tests, baking temperatures, fat levels, kinds of fat, and the interaction between them.

Individual records were kept of the gains in weight and feed consumption for the 28-day feeding periods. During the fourth week of each growth test, faeces were quantitatively collected from each rat, air-dried, and stored, pending chemical analysis, so that apparent digestibility coefficients for the diet and diet fractions could be determined.

To obtain a measure of the metabolic faecal fat for true digestibility of the shortenings the data from the rats receiving the check diets of 0% fat were

used. The average daily fat output on the fat-free diet was found to be 0.15 gm. The true digestibility of fat was calculated as:

$$100 \times \left(\frac{(\text{Dietary fat intake}) - (\text{Fat output} - \text{average metabolic fat})}{\text{Dietary fat intake}} \right)$$

At the conclusion of the growth trial all rats were killed by stunning. The carcasses and livers were analysed for crude fatty acids by the method of Gavin and McHenry (6, 13).

Results

Because of the relation of digestibility and of carcass and liver fat deposition data to the interpretation of the growth results they will be considered first.

The mean values of the digestibility coefficients for the total diet and the energy yielding fractions are given in Table II.

TABLE II
MEAN VALUES OF PERCENTAGE DIGESTIBILITY OF DIETS AND DIET FRACTIONS

Variable	Group	% Digestibility, (to nearest whole per cent)				
		Total dry matter	Carbo- hydrate	Protein	Fat	
					Apparent*	True
Baking temperature, °F.	375	93	96	84	96	97
	425	93	95	83	97	98
Kinds of fat	Animal-veg.	92	95	83	95	97
	Veg. blend	93	96	84	96	98
	Hydrog. veg.	92	95	84	96	97
	Animal (lard)	94	96	83	97	99
Fat level, %	0	95	98	86		
	8	94	97	85	96	97
	16	92	95	82	96	97
	24	91	92	81	97	98
Test averages		93	96	83	96	97

* Necessary difference for significance ($P = 0.05$).
 Between baking temperatures = 0.5
 Between kinds of fat = 0.7
 Between levels of fat = 0.6

It is evident that baking temperature did not affect digestibility. Lard appears to have a slightly higher digestibility coefficient than the other three shortenings. This finding is in accordance with that of Hoagland and Snider (8) who reported that the digestibility of lard was superior to that of other types of shortenings. The difference is small, however, and probably of no practical consequence. For reasons not explainable in this test the apparent digestibility of the total dry matter, carbohydrate, and protein decreased and that of fat increased as the fat content of the diet increased, while the true digestibility of the fat remained constant.

In so far as the carcass and liver analyses are concerned, no pathological evidence of fatty livers was observed in any of the rats nor was there any evidence of differences in fat deposition in the carcasses except in the case of lard, which showed low values. The data are given in Appendix Table I.

In Table III the effect of the two baking temperatures on the fat deposition in rats fed the four different dietary fats is given.

TABLE III

EFFECT OF TWO BAKING TEMPERATURES ON THE CARCASS FAT DEPOSITION OF RATS FED FOUR DIFFERENT FATS

(Figures are % fatty acids in carcass)

Baking temp., °F.	Kind of fat			
	Animal-vegetable	Blended vegetable	Hydrogenated vegetable	Animal (lard)
375	8.4	7.1	8.3	4.8
425	6.0	6.4	6.0	2.9

The subnormal deposition of fat in the lard-fed rats would seem to be due to the greater adverse effect of heat on lard than on the other three shortenings. The lard diets were the only ones obviously rancid during the test. This might account for the lower fat deposition in rats fed the lard diets. Furthermore, as high temperatures are known to speed up the onset of rancidity, it is probable that this was the cause for the decreased fat deposition with the increased baking temperature.

Growth

A summary of the average initial weights of the rats, their 28-day gains in live weight and their feed consumption is presented in Appendix Table II.

In order to distinguish between differences in gains due to kinds of feed and those due to amounts of feed, the observed gains were adjusted by regression ($b = 0.299$) to average feed intake. The mean observed gains, feed intakes, and adjusted gains are given according to feeding groups in Table IV, from which the following observations can be made.

Baking Temperature

Animals on the diets baked at 375° F. showed significantly greater gains than those fed diets baked at 425° F. This, however, does not seem to be due to any effect of heat on the fat, since the greatest decrease in gains on the higher temperature occurs when there is no fat in the diet (See Table V). The inferior gains on diets baked at 425° F., compared with gains on diets baked at 375° F., therefore, would seem more likely to be due to damage to the protein at the higher temperature. This is in accord with the evidence of Morgan and King (15) who report retarded growth of rats receiving toasted bread as compared with controls fed bread crumbs.

TABLE IV
MEAN 28-DAY GAINS AND FEED INTAKE ACCORDING TO FEEDING GROUPS
(Figures to nearest gram)

Variable	Group	Observed 28-day gains	28-day feed intake	Adjusted 28-day gains	Necessary difference ($P = 0.05$)
Baking temperature, °F.	375	47	215	47	1.5
	425	43	210	44	
Kind of fat	Animal-veg.	50	226	46	2.1
	Veg. blend	43	204	46	
	Hydrog. veg.	54	231	48	
	Animal (lard)	35	190	42	
Fat level, %	0	77	297	52	2.1
	8	41	203	44	
	16	36	186	44	
	24	28	166	42	
Test average		45	213	46	

TABLE V
EFFECT ON GAINS OF DIFFERENT BAKING TEMPERATURES ON DIETS
CONTAINING DIFFERENT LEVELS OF FAT
(Figures to nearest gram)

Baking temp, °F.	Fat level			
	0%	8%	16%	24%
375	56	43	44	44
425	47	45	44	40

Kinds of Fat

Diets containing the pure animal fat (lard) produced gains inferior to those produced by any one of the other three shortenings. The lower feed intake suggests a lack of palatability of the lard diets. In this connection it may be noted that the diets with lard showed an easily detected rancidity before the feeding tests were completed. Clausen, Barnes, and Burr (1) stressed the seriousness of destruction of dietary essentials and possible toxicity of rancid fats. In their studies, when lard was included in the diet with no antioxidant, rats failed to grow, and died, but if the lard were replaced by hydrogenated vegetable oils, corn oil, or butterfat, growth occurred normally.

Fat Level

Between levels of fat there are striking differences in gains as shown in Table IV. The addition of fat was associated with a sharply reduced feed consumption. We believe this to be related to the effect of fatty meals in

delaying gastric emptying time. On *ad libitum* feeding the animals eat when hungry, and probably the slower emptying time of the stomach on fatty diets decreases the frequency of hunger and thus may decrease the feed intake per day. Thus, indirectly, the addition of fat to the diet appears to have caused a drop in gains. It should be noted, however, that the efficiency of the fat-containing diets, though the same for the three levels, is below that of the fat-free ration as measured by the gains on equal feed intake.

The results of the fat level comparisons may perhaps be more clearly presented if the feed intake is expressed in terms of calories of metabolizable energy. The metabolizable energy of each diet is presented in Appendix Table III, and was determined from the composition of the diet in energy yielding fractions, and the digestibility of these components, using heats of combustion of 4.1 Cal. per gm. for carbohydrate, 9.35 for fat, and 5.65 for protein with an assumed urine calorie loss of 1.25 Cal. per gm. of digestible protein (Maynard (14)).

The metabolizable energy per 100 gm. of diet, the mean gains, the average feed intake and its equivalent in metabolizable energy, and the calories required per gram of gain, are given in Table VI.

TABLE VI

GAINS, FEED INTAKE, METABOLIZABLE ENERGY, AND CALORIES PER GRAM GAIN

Fat in diet, %	Average gain	Average feed consumed	Metabolizable* energy, Cal./100 gm.	Calories of metabolizable energy consumed	Calories per gram of gain
0	77	297	380	1129	14.7
8	41	203	417	847	20.7
16	36	186	452	841	23.4
24	28	166	487	808	28.9

* Metabolizable energy for each diet given in Appendix Table III.

From this table it will be seen that, as the level of fat increases, the metabolizable energy per 100 gm. of diet has increased (Column 4). But the feed intake (Column 3) has declined sufficiently to cause an actual decline in gains. The feed efficiency (Column 5), however, has also declined, so that the energy needed per unit gain (Column 6) has steadily increased with rise in fat level.

Multiple correlations indicated that about 87% of the variability in gains was traceable to variations in the intake of calories of metabolizable energy from protein, from carbohydrate, and from fat. Partial correlation indicated that the proportion of calories from protein was the principal dietary factor involved in these results. Indeed β -values* suggested that 70% of the effect of the three energy yielding diet fractions was chargeable to protein calories

* Standard partial regression coefficient. See "Correlation and machine calculations", Wallace and Snedecor. Iowa State College of Agriculture and Mechanic Arts, Ames, Iowa.

and only 5% to the fat level. The partial regression b^* of protein calories on gains was 0.91; of carbohydrate, 0.07; and of fat 0.03 gm.

In Fig. 1 is shown the extent to which the proportion of total calories from protein decreased with increased fat level, and its relation to 28-day gains of the rats. It will be noted that on a weight basis, the level of protein was constant at 16%.

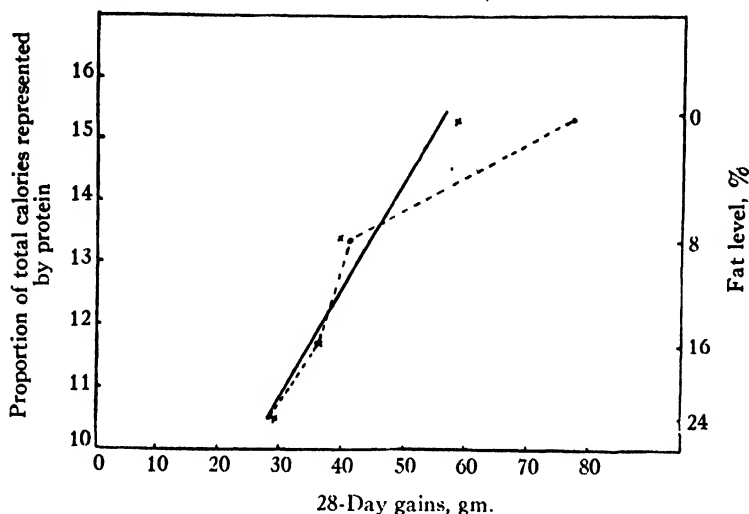


FIG. 1. Relation of proportion of total calories represented by protein, gain, and fat level. ----- Observed gains. ————— Gains adjusted to average calories of fat diets.

From this graph it is evident that the trend of increasing gains with increasing proportion of protein calories is practically linear between the fat containing diets, but departs significantly from this trend between the diet with lowest fat (8%) and the fat-free diet. This is traceable almost entirely to the sharply increased feed intake in the lot on the fat-free diet. Comment on the depressing effect of fat on feed consumption has already been made. If the effects of feed intake are taken into account by calculating the gain made per 832 Cal. of food (the average caloric intake of the animals on fat-containing diets) this departure from linearity disappears as shown by the broken line in Fig. 1.

Levine and Smith (11) report that as long as the proportion of protein calories to total calories was kept at about 14%, normal growth could be obtained on a high fat diet (86% of total calories).

The data of this test emphasize again the importance of balance in the ration between protein and non-protein fractions in determining the nutritive value of a diet.

Protein may be raised above minimum requirements at the expense of carbohydrate without necessarily causing any change in the energy value of

$$* b_{ax} = \beta_{ax} \left(\frac{\sigma \text{ of dependent variable, } x}{\sigma \text{ of independent variable, } a} \right).$$

the diet or in resulting gains. However, if the percentage of fat is increased at the expense of protein or carbohydrate, or both, on a weight basis, the energy value of the diet is raised, but in addition the proportion of calories from protein may be decreased below that necessary for maximum nutritive value.

Summary

1. In these tests, digestibility of the fat of the diets was unaffected by baking temperature or by level of shortening in the rations. Lard was very slightly more digestible than the types that included vegetable fats. The range in coefficients was from 95.4 to 97.4% for apparent, and from 96.5 to 98.7% for true, digestibility.

2. Excepting for lard, neither type nor level of fat, nor baking temperature affected appreciably the deposition of fat in the carcasses. No evidence of fatty livers was found. Lard-fed rats showed considerably lower than average carcass fat deposition and this effect was more pronounced at the higher baking temperature. These results may reflect the effect of marked rancidity that developed with the lard.

3. Rats fed diets baked at 425° F. made considerably slower gains than those on the diets baked at 375° F. This was not traceable primarily to heat damage to the fat but was more probably the result of damage to some protein fraction.

4. Lard diets were least well eaten, with the result that they produced the lowest gains. This is probably the result of the fact that the lard became rancid.

5. Food intake was reduced by the inclusion of fat in the diets. This is believed to be due to the depressing effect of fatty meals on gastric emptying time and consequently to a reduction of the frequency of eating.

6. Observed gains and gains per unit of food intake progressively decreased with increasing fat level of the diet. This was correlated with a reduction in the proportion of calories from protein to non-protein sources, resulting from the replacement of carbohydrate yielding 4 Cal. with fat yielding about 9 Cal. per gm. Protein level on a metabolizable calorie basis may be of more nutritional significance than protein level on a weight basis if variation in fat content of the diet is involved. This in effect means that as the fat level is increased in a diet, the percentage protein, on a weight basis, must also be increased to maintain the minimum protein-non-protein calorie ratio for maximum nutritive value.

Acknowledgments

This project was made possible through financial assistance from the Associate Committee on Army Medical Research of the National Research Council of Canada. The authors wish to acknowledge also the assistance of Harrison Brothers Limited, Montreal, who did all the baking involved in this project. They also wish to thank the Swift Canadian Company and Lever Brothers Limited for furnishing samples of shortenings for this test.

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APPENDIX TABLE I

MEAN VALUES FOR PERCENTAGE CRUDE FATTY ACIDS OF LIVERS AND CARCASSES

Variable	Group	Livers		Carcasses	
		Crude fatty acids, %	Necessary difference $P = 0.05$	Crude fatty acids, %	Necessary difference, $P = 0.05$
Baking temperature, °F.	375	2.05	0.29	7.17	0.88
	425	1.73		5.33	
Kind of fat	Animal-veg.	1.63	0.41	7.21	1.25
	Veg. blend	1.68		6.74	
	Hydrog. veg.	2.15		7.15	
	Animal	2.10		3.88	
Level of fat, %	0	1.30	0.41	6.15	1.25
	8	1.88		6.35	
	16	2.13		6.45	
	24	2.25		6.03	
Test averages		1.89		6.25	

APPENDIX TABLE II

MEAN VALUES FOR INITIAL WEIGHTS, 28-DAY GAINS, AND FEED CONSUMPTION (gm.)

Baking temp., °F.	Fat level, %	Variable recorded	Kind of fat			
			Animal-vegetable	Blended vegetable	Hydrogenated vegetable	Animal
375	0	Initial weight 28-day gain 28-day feed consumption	51 81 285	54 94 338	51 78 284	53 79 298
	8	Initial weight 28-day gain 28-day feed consumption	53 55 237	57 37 202	51 49 206	49 17 141
	16	Initial weight 28-day gain 28-day feed consumption	54 46 214	50 28 162	47 43 194	55 27 174
	24	Initial weight 28-day gain 28-day feed consumption	55 36 198	48 34 170	56 42 203	46 16 121
425	0	Initial weight 28-day gain 28-day feed consumption	52 80 317	49 69 273	56 73 310	44 64 253
	8	Initial weight 28-day gain 28-day feed consumption	44 38 185	50 46 215	51 57 241	48 28 172
	16	Initial weight 28-day gain 28-day feed consumption	58 41 217	45 19 133	51 51 214	52 32 182
	24	Initial weight 28-day gain 28-day feed consumption	51 27 161	51 16 140	47 36 181	58 17 155

APPENDIX TABLE III

METABOLIZABLE ENERGY OF DIETS

Baking temp., °F.	Fat level, %	Type of fat			
		Compound animal-vegetable	Blended vegetable	Hydrogenated vegetable	Animal
375	0	380*	382	381	381
	8	417	411	418	428
	16	451	445	451	466
	24	481	483	483	506
425	0	380	379	379	379
	8	415	413	416	421
	16	446	448	449	460
	24	483	482	482	494

* Cal./100 gm. diet.

DRIED WHOLE EGG POWDER

XIX. ACCELERATED STORAGE TESTS TO ASSESS THE EFFECT OF HEAT TREATMENT, MOISTURE CONTENT, AND MATERIALS ON THE QUALITY OF DRIED SUGAR-EGG MIXTURES¹

BY R. L. HAY² AND JESSE A. PEARCE²

Abstract

Deterioration in quality was assessed by fluorescence, potassium chloride, pH, and foaming volume measurements.

Dried egg powder (moisture content, 2.8%), containing 33% sugar, and control samples of plain egg powder (moisture content, 3.9%) were stored at temperatures of 80°, 100°, 120°, and 140° F. for seven days. At 140° F. the addition of sugar inhibited the initial, but not the secondary, fluorescence development observed in the plain egg powder and retarded deterioration as assessed by other measurements. At temperatures of 120° F. and lower, the presence of sugar had a marked effect in retarding decrease in quality in egg powder as assessed by all quality tests used. Interpretation of the results in terms of commercial drying practices indicated that cooling shortly after drying was less important for sugar-egg powder than it was for plain egg powder.

Dried egg powder containing 33% sugar was adjusted to moisture levels of 1.4, 2.8, and 3.2% and stored at 80° and 120° F. for seven days. The rate of deterioration in quality of sugar-egg powder increased markedly with both moisture content and temperature. Egg powder containing 1.4% moisture maintained higher quality at both temperatures for a longer period than powders at either 2.8 or 3.2% moisture levels. It is recommended that sugar-egg powder be dried to the lowest moisture content compatible with the production of good quality powder, certainly to a moisture content of less than 2.8%, and preferably to 1.4%.

Loss in quality was less for sugar-egg powders (moisture content, approximately, 2.3%) prepared with granulated sugar than for those prepared with sucrose syrup, when stored at 80°, 100°, 120°, and 140° F. for seven days. In addition, powder made from fresh shell eggs was more desirable than powder prepared from frozen melange. It is recommended that sugar-egg powder be prepared from a mixture of sugar in granulated form and melange from fresh shell eggs.

Introduction

Eggs in powdered form have become a well known commodity during the war years. The production of egg powder in Canada during pre-war years was almost negligible but it is probable that the demand for this product will be increased during the post-war period. However, the extent to which this commodity competes successfully with other egg products during normal times will depend largely on how well its quality can be maintained, not only during production but also during subsequent handling and storage.

In a recent communication it was observed that the addition of sucrose to egg powder, prior to drying, was effective in delaying fluorescence development at temperatures of 118° F. and lower (9). Present indications are that this sugar-egg powder will find a ready peacetime market for baking and other trade purposes.

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Previous investigations have shown that temperature and moisture content were major factors in the preservation of dried foods. Earlier studies showed that rapid cooling of plain egg powder to a temperature of 80° F. or less within three hours of leaving the drier was important if high quality were to be maintained (14, 15, 16). Lowering the moisture content prolonged the storage life of this powder (7, 15) but even at the low level of 1.4% moisture, dried egg deteriorated in a relatively short time when stored at temperatures of 99° and 118° F. (13). Moisture levels of 2% and less were believed desirable, but Canadian commercial drying operations did not permit the attainment of these lower levels.

Some sugar-egg powder is being produced in Canada at the present time. Current drying practice favours the production of sugar-egg from melange to which sucrose syrup rather than granulated sucrose has been added, although no comparison of the relative qualities of the final products has been made. Furthermore, no attempt has yet been made to compare the quality of sugar-egg powder prepared from shell eggs with that prepared from frozen melange.

Since sugar in egg had an inhibitory effect on fluorescence development, it was of interest to determine whether sugar-egg was as susceptible to heat deterioration as plain egg powder, to compare the effect of various moisture contents on the quality of sugar-egg powders, and to make some assessment of the function of sugar in this product. This report also describes the effect of heat treatment on sugar-egg powders produced from the following: (a) shell eggs and sucrose syrup, (b) shell eggs and granulated sucrose, (c) frozen melange and sucrose syrup, and (d) frozen melange and granulated sucrose.

Comparisons of plain and sugar-egg powder in this study were made on the assumption that the moisture was almost entirely contained in the egg fraction of the sugar-egg. Reference to equilibrium relative humidity data for egg powder showed that, at room temperature and about 3% moisture, the relative humidity over a sample of plain egg powder would be about 15% (5). Reference to similar data (4) for sugar at 25° C. indicated that commercial sucrose (containing 0.05 to 0.2% invert sugar) would not begin to take up moisture until a relative humidity of about 70% was reached. Even if inversion occurred during the drying process, it is unlikely that moisture would be elsewhere than in the egg powder, since sucrose containing 10% invert sugar does not begin to take up moisture until a relative humidity of about 25% is reached.

Materials and Methods

The powder containing 33% sugar (dry matter basis) and the plain egg powder used in the heat treatment study were obtained from the same commercial Canadian source. These samples were tempered in the laboratory to a moisture content of approximately 4%, calculated on the basis of egg solids. The actual moisture content of the sugar-egg was 2.8%; the plain egg, 3.9%. The sugar-egg powder used in the moisture study was obtained from the same Canadian producer, and was tempered to moisture levels of 1.4, 2.8,

and 3.2%. Calculated on the basis of egg solids, the moisture contents were 2.1, 4.0, and 4.6% respectively. After the moisture adjustment had been completed samples of these powders were packed in tin-plate (air as headspace gas), stored at temperatures of 80°, 100°, 120°, and 140° F. and removed for analysis after one, two, five, six, and seven days.

The powders prepared from various mixtures (sugar content, 33%, dry weight basis) were obtained from another Canadian producer and adjusted in the laboratory to approximately the same moisture level (actual moisture content between 2.2 and 2.4%). Samples of these powders were also packed in tin-plate (air as headspace gas), stored at 80°, 100°, 120°, and 140° F., and removed for analysis after one, two, three, four, five, six, and seven days.

All powders were prepared on a cone type spray-drier. The choice of producers was a matter of convenience only. The drying temperatures were those believed most desirable for the respective pieces of equipment.

The quality of the powders was evaluated by measurement of fluorescence (8), potassium chloride value (12), pH (12), and foaming volume; the last was believed to provide a method of assessing the baking quality. The procedure for measuring the foaming volume of the plain egg samples was the same as that reported in an earlier study (11). For the sugar-egg samples, the foaming volume was assessed as follows: 40.5 gm. of the powder was mixed thoroughly with 75 ml. of distilled water. Best results were obtained by adding a small amount of the water to the powder, whipping the mixture manually into a thick homogeneous paste, and then pouring in the remainder of the water. The mixture was beaten at the highest speed in a "Mixmaster" for 10 min., and the volume of the foam measured in a graduate. Although the two methods used were not comparable, it was felt that relative differences occurring during storage would be apparent.

Results

The Effect of Heat Treatment

The relative effects of temperature and storage time on the quality of both types of egg powders were evaluated by means of analyses of variance. The significant results are shown graphically in Figs. 1a and 1b. Fluorescence values increased and potassium chloride values, pH values, and foaming volume decreased with both storage temperature and time.

At least two separate reactions may occur during the development of fluorescing materials in plain egg powder, since the previous curves have shown a definite break or irregularity during the formation of fluorescing substances (14). This has suggested the possible presence of an initial and a secondary reaction. In the present study, curves for the fluorescence development in materials containing no sugar showed evidence of two reactions, but the presence of sugar appeared to retard the initial reaction.

At temperatures of 120° F. and lower, sugar had a marked effect in reducing the rate at which fluorescence developed. Sugar-egg at 140° F. attained the

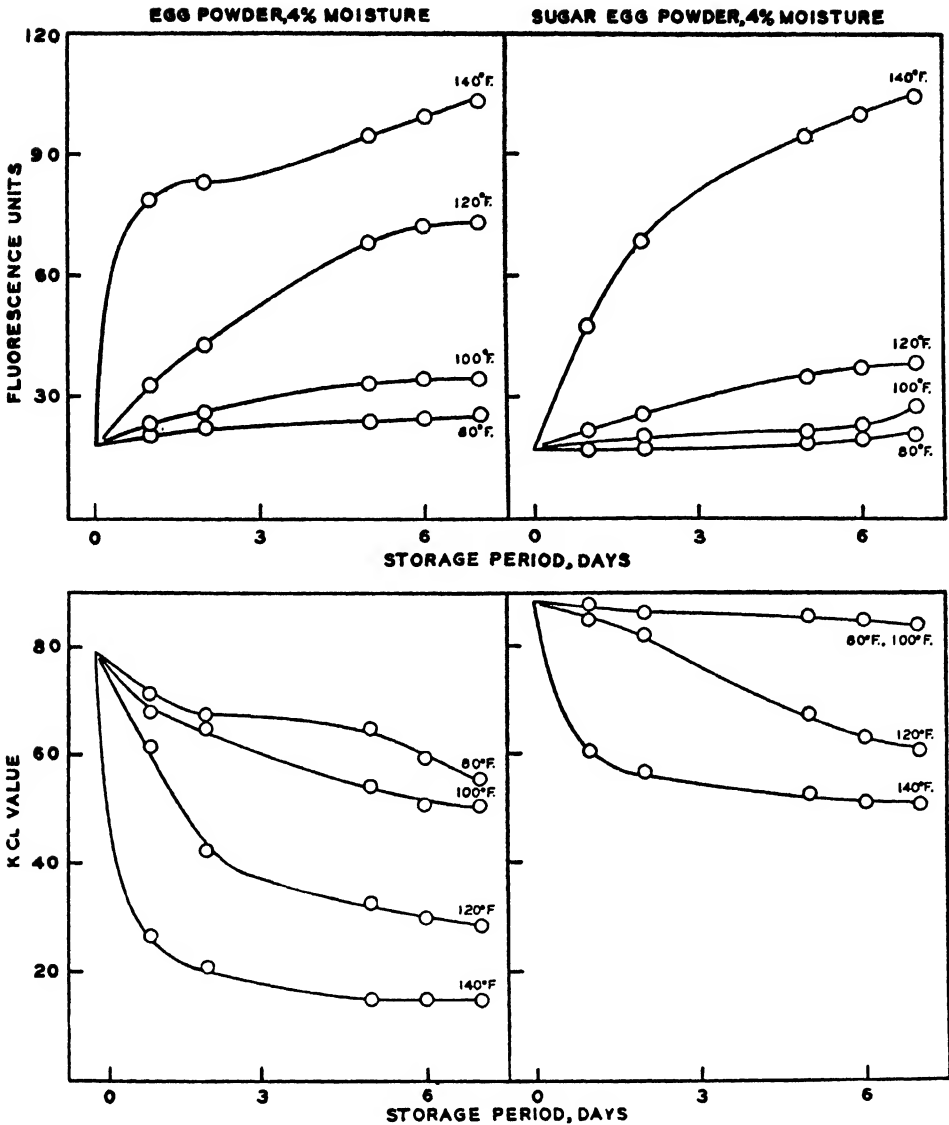


FIG. 1a. Effect of heat treatment on fluorescence values and potassium chloride values of plain and sugar-egg powders with a moisture content of 4%, calculated on the basis of egg solids present. (Actual moisture content of sugar-egg powder, 2.8%.)

same maximum value as plain egg, but the curve indicated retardation, elimination, or alteration of the first stage of the fluorescence reaction (Fig. 1a). At 140° F., the presence of sugar reduced the development of fluorescence by about one-half after storage for one day.

Earlier work had shown that sugar reduced fluorescence development during the first two weeks' storage at 118° F. and during eight weeks' storage

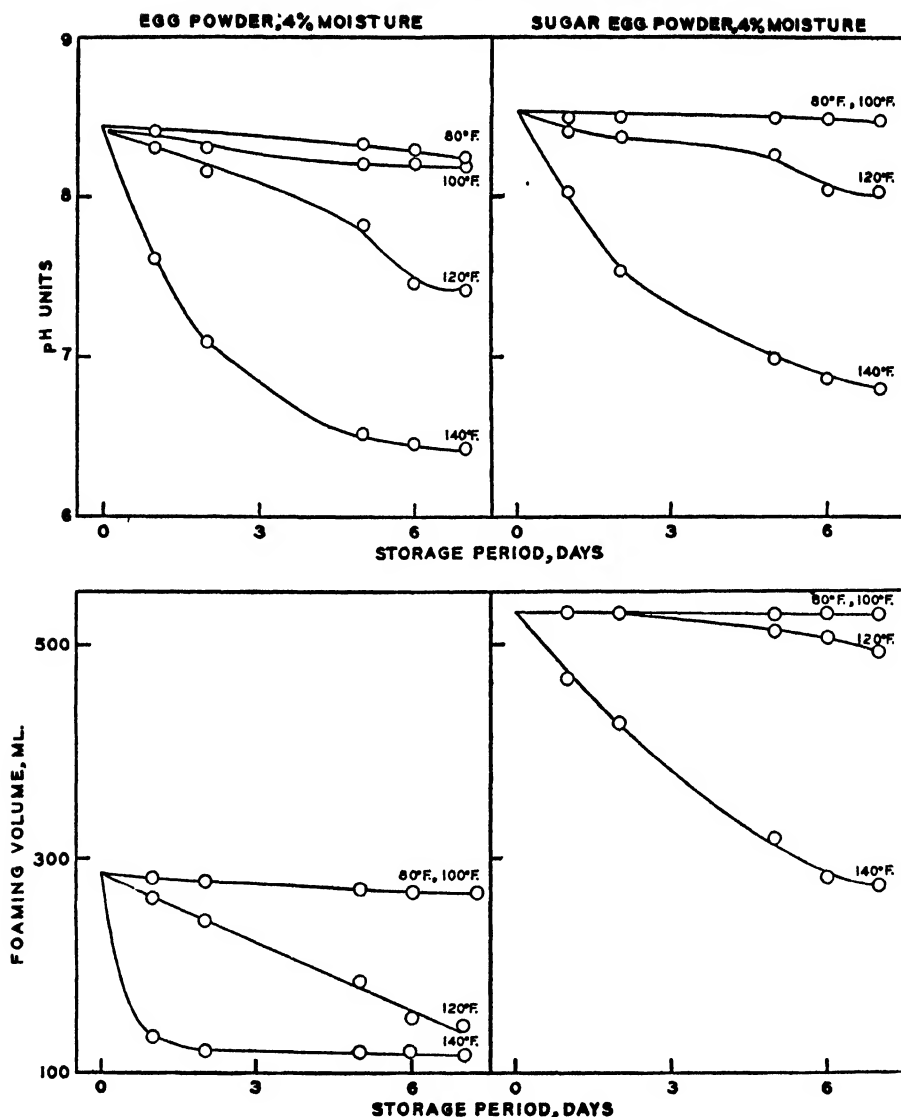


FIG. 1b. Effect of heat treatment on pH and foaming volume of plain and sugar-egg powders with a moisture content of 4%, calculated on the basis of egg solids present. (Actual moisture content of sugar-egg powder, 2.8%).

at lower temperatures (9). The present study showed that sugar effectively retarded fluorescence development during seven days' storage at 80°, 100°, and 120° F.

The maximum fluorescence value allowable for Canadian Grade A plain egg powder has been established at 24, and that for Grade B at 57 (3). Prime quality sugar-egg powder stored at 80° F. remained at a lower fluorescence level than that of plain egg during the entire period of seven days (Fig. 1a).

At 100° F., high quality was maintained in the sugar-egg five days longer than in the non-sugar powder. Best quality sugar-egg powder stored at 120° F. decreased to Grade *B* quality in 48 hr., while only about eight hours were required for a similar deterioration in plain egg powders. At 140° F., sugar appeared to have an inhibitory effect sufficient to delay deterioration for several hours.

Fluorescence development as depicted in Fig. 1*a* revealed that sugar added to egg powder before drying provided a beneficial effect on quality. Since sugar-egg powder was less susceptible to heat treatment it appeared that cooling to 80° F. shortly after drying would not be as important for sugar-egg as it is for plain egg.

The solubility of plain egg powders in a 10% potassium chloride solution decreased when either temperature or storage time was increased, and this was believed due to thermal decomposition of a fat-protein complex and denaturation of the egg protein (14).

In this study (Fig. 1*a*), the solubility of plain egg decreased during storage at all temperatures, the most marked change occurring in the first two days at 140° and 120° F. Subsequent changes at these temperatures were relatively slow; this indicated that the denaturation processes may have been approaching completion. The presence of sugar in egg powder appeared to provide the most protection at 80° and 100° F., and a comparison of the curves for sugar-egg and plain egg powders at 120° F. also showed evidence of some preservative action.

Correcting the potassium chloride values of sugar-egg powder for the presence of added sugar indicated that about 80% of the egg solids were soluble, which is about the same quantity usually soluble in fresh plain egg powder. After two days at 140° F., plain egg powder was only 20% soluble, but the egg fraction of sugar-egg powder was still about 40% soluble. This indicated that the presence of sugar in some way retarded or impeded thermal decomposition of the fat-protein complex or protein denaturation.

It has been reported that a decrease in pH accompanied quality deterioration in egg powders (12, 14). Further evidence of this is apparent in Fig. 1*b*. The pH of the plain egg powder decreased at all temperatures, with the greatest reduction occurring during the first five days at 140° F. The stabilizing effect of sugar as indicated by an almost constant pH was most evident at temperatures of 80° and 100° F. Sugar had a slightly beneficial effect at 120° F., but less at 140° F. These observations agreed with, and supported, the results obtained from the more sensitive fluorescence and potassium chloride tests.

For the plain egg powder the decrease in foaming volume at both 80° and 100° F. was relatively small, and of approximately the same order of magnitude. At 120° F. the decrease in this attribute was greater than at the lower temperatures, but the greatest change occurred in the plain egg during the first 24 hr. at 140° F. The subsequent changes at the high temperature were extremely slow; this indicated that the components responsible for the foaming property had been altered rapidly.

Although the techniques for determining foaming volume differed for plain and sugar-egg, and a marked increase in foaming volume is known to result from the addition of sugar to egg powder prior to drying, it was felt that the relative changes noted here were comparable (1). At 80° and 100° F. the foaming volumes of sugar-egg remained approximately equal to the initial value during the entire storage period. At 120° F. the foaming volume of sugar-egg powder decreased only 46 ml. after seven days in storage. Under the same conditions, the foaming volume of the plain egg powder decreased 145 ml. Thus, sugar had marked effect in maintaining high foaming volumes at 120° F. At 140° F. total loss of the components responsible for the foaming quality appeared to have been postponed several days owing to the presence of sugar.

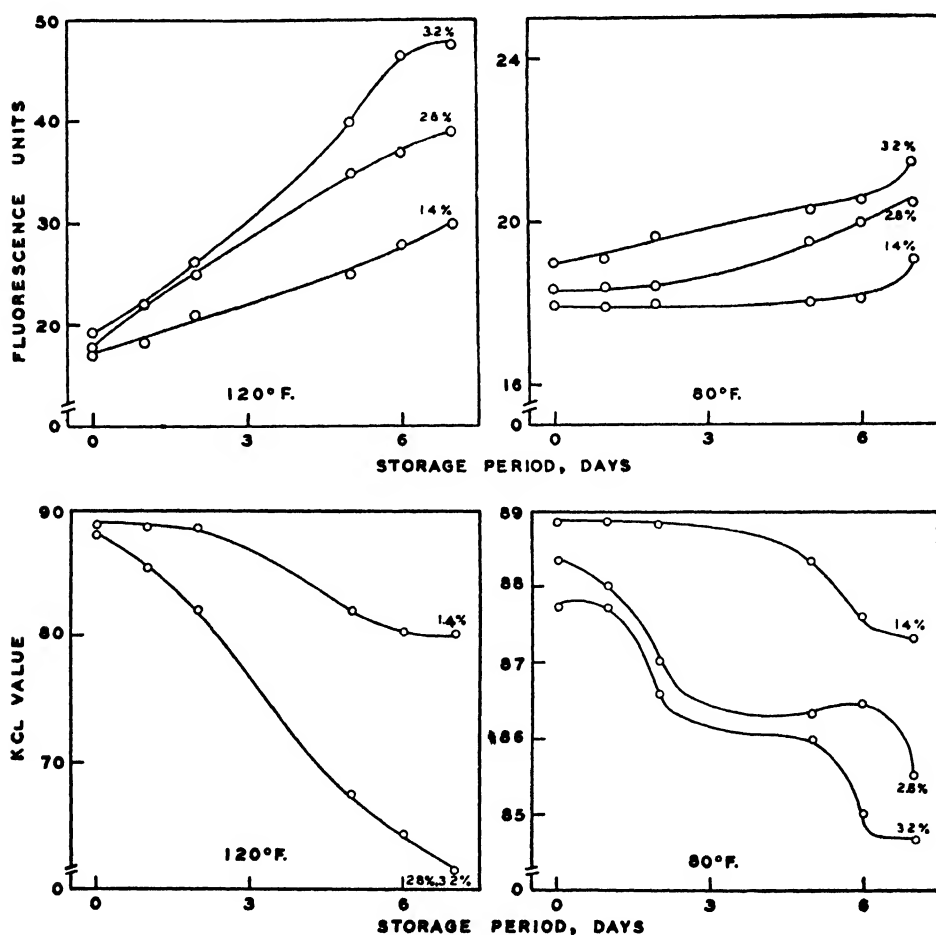


FIG. 2a. Effect of heat treatment on fluorescence values and potassium chloride values of sugar-egg powder having actual moisture contents of 1.4, 2.8, and 3.2%.

The Effect of Moisture Content

The effect of moisture content on the quality of sugar-egg powders was also evaluated by means of analyses of variance, and the significant results are shown graphically in Figs. 2a and 2b. The general effects of temperature and storage time noted in the heat treatment study were again observed in this study. Powder containing 1.4% moisture was better initially, except for foaming volume, and remained superior to the other powders during storage. The 3.2% powder produced the highest initial foaming volume but after the sixth day in storage deteriorated to a level below that of the lower moisture powders.

The rate at which the fluorescence value increased was roughly proportional to the moisture content. At 80° F., egg powder with a 3.2% moisture

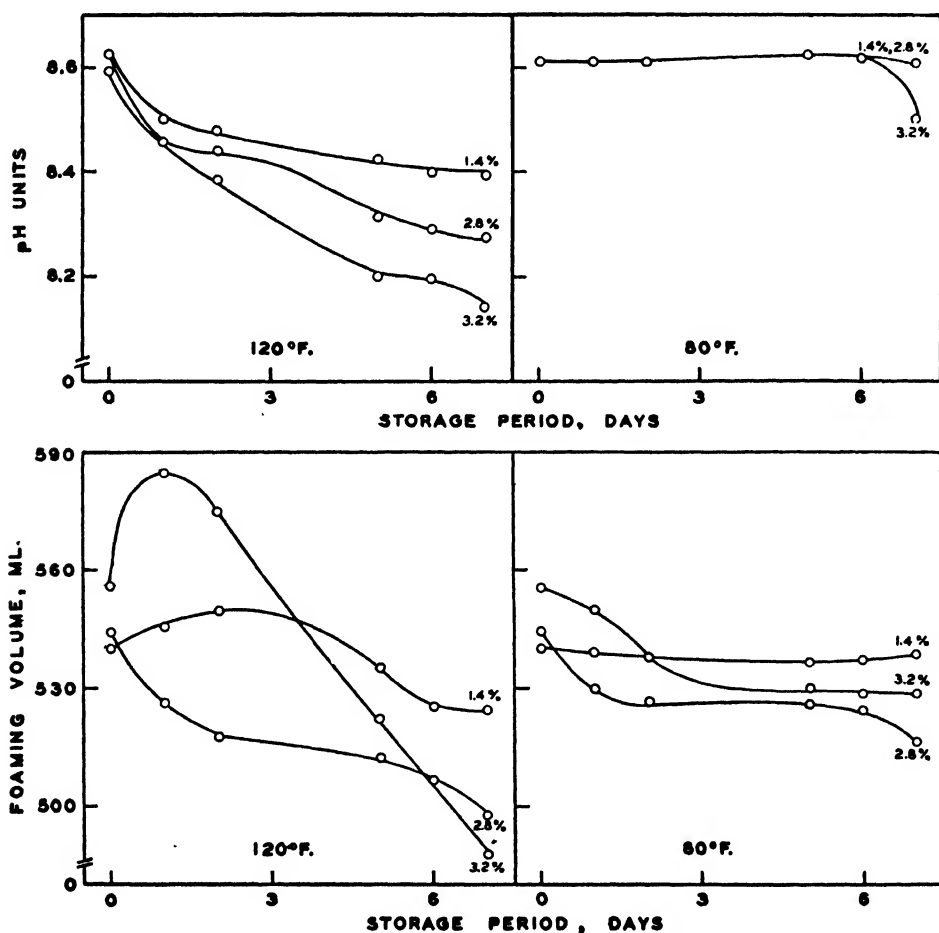


FIG. 2b. Effect of heat treatment on pH and foaming volume of sugar-egg powder having actual moisture contents of 1.4, 2.8, and 3.2%.

content remained at a higher fluorescence level than either the 1.4 or 2.8% powders throughout the storage period of one week. At 120° F., powder containing 1.4% moisture had an initial fluorescence value of 17 and this increased linearly to a final value of 30 after the seventh day of storage. The 2.8 and 3.2% powders deteriorated at approximately the same rate during the first few days but attained final fluorescence values of 39 and 48, respectively. The 2.8 and 3.2% powders had attained a fluorescence value of 24, the maximum value allowable for Canadian Grade A egg powder (3), after two days of storage at 120° F., while five days were required for a similar deterioration in the 1.4% powder; this indicated that drying sugar-egg to a low moisture content might extend the life of the product about two and one-half times.

Egg powder containing 1.4% moisture and stored at 80° F. for seven days had a higher solubility than either the 2.8 or 3.2% powders. The solubility of the egg powders at all three moisture levels decreased more rapidly during storage at 120° F. than during storage at 80° F., with the most marked decreases occurring in powders with moisture contents of 2.8 and 3.2%. A comparison of the curves (Fig. 2a) for powders stored at 120° F. showed that loss in solubility in the 1.4% powder was approximately one-third that in the 2.8 and 3.2% powders after storage for one week.

Sugar-egg powders containing 1.4% moisture maintained higher pH values for a longer period than those powders at either 2.8 or 3.2% moisture levels (Fig. 2b). At 120° F., the decrease in pH was most rapid for the powder with 3.2% moisture. The powder with 1.4% moisture maintained the highest pH level during the entire storage period.

These results of foaming volume measurements on the stored powders were slightly different from those shown by the previous measurements. During the first few days 3.2% moisture appeared to be most desirable but after seven days the foaming volumes of this powder had decreased rapidly to a level below that of the 1.4 and 2.8% powders. The curves indicated that this initial beneficial effect was of short duration and that after an extended storage period the powder containing the lowest moisture would be most desirable. Extended studies on the change in foaming volumes of sugar-egg powders are currently under investigation in these laboratories.

Effect of Materials Used

The effects of temperature, storage time, method of adding sugar, and prior condition of melange, on quality of the sugar-egg powder were also evaluated by means of analyses of variance. Although initially the powders did not differ in quality, every test used on the stored samples showed significant differences to result from the different materials used (Tables I and II). Differential behaviour of significance is shown in Figs. 3 and 4. Both the tables and the figures contain mean values of each variable, as calculated over all others for the whole experiment.

TABLE I

EFFECT OF HEAT TREATMENT ON SUGAR-EGG POWDERS PREPARED FROM SHELL EGGS AND FROM FROZEN MELANGE

Testing method	Mean value for powder prepared from			
	Shell eggs		Frozen melange	
	Initially	After storage*	Initially	After storage*
Fluorescence value	20.0	30.2	22.2	27.4
Potassium chloride value	86.4	79.3	87.3	77.8
Foaming volume	632	611	602	568
pH value	8.64	8.42	8.57	8.36

* Averaged over all storage times and temperatures.

TABLE II

EFFECT OF HEAT TREATMENT ON SUGAR-EGG POWDERS PREPARED WITH SUGAR AND WITH SYRUP

Testing method	Mean value of powder prepared with			
	Granulated sugar		Syrup	
	Initially	After storage*	Initially	After storage*
Fluorescence value	20.5	28.2	21.7	29.6
Potassium chloride value	87.3	79.2	86.0	77.9
Foaming volume	638	623	600	556
pH value	8.64	8.44	8.56	8.34

* Averaged over all storage times and temperatures.

The rate of deterioration in all powders increased with both temperature and storage time (Fig. 3), and the trends observed were similar to those noted in the earlier study (Figs 1a and 1b). Comparison of the curves in Fig. 3 with those in Figs. 1a and 1b shows the beneficial effect of a reduction of 0.5% in moisture content.

The initial measurements showed small differences between the various samples of the dried product, and all but the fluorescence measurements on the stored powders supported the evidence that shell egg produced a better dried material than frozen melange (Table I). However, another investigation in these laboratories has shown that freezing reduces the fluorescence of liquid egg (10) and also reduces the intensity of the light given off by fluorescing materials isolated from egg powder (2). Therefore, the low fluorescence values for stored powders prepared from frozen material do not necessarily indicate high quality. The superiority of shell egg over frozen egg was shown most markedly by potassium chloride and foaming volume measure-

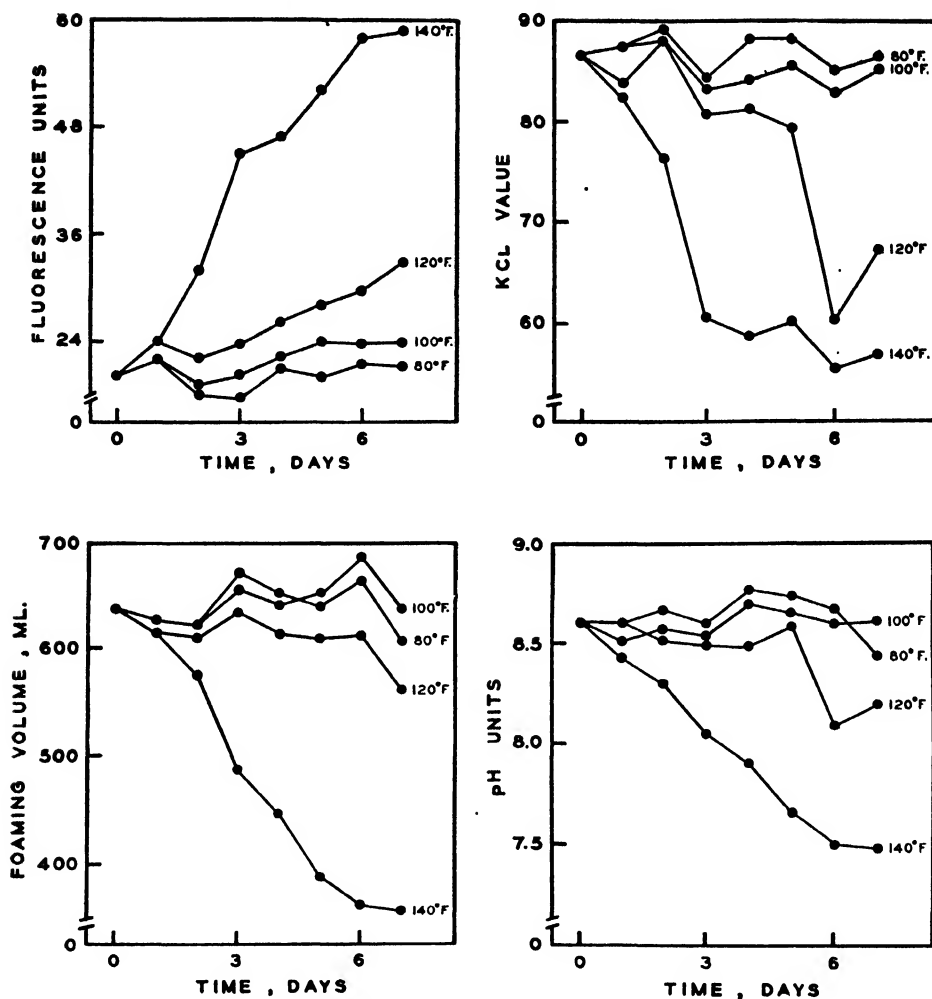


FIG. 3. Average effect of heat treatment on fluorescence values, potassium chloride values, pH, and foaming volume of sugar-egg powder prepared from shell and frozen eggs using sucrose crystals and sucrose syrup. Actual moisture content of these samples was about 2.3%. Comparison with Fig. 1a indicates the improvement resulting from 0.5% reduction in moisture content.

ments. Freezing the melange apparently made some of the soluble constituents, possibly those responsible for the aerating properties in egg, less stable. This is receiving further consideration in these laboratories.

Sugar-egg powder prepared by adding sugar appeared slightly better initially than the product prepared from syrup, and when the powders were stored these differences became significant as assessed by all measurements (Table II). The initial 38 ml. difference in foaming volume values was considered most important, especially as sugar-egg is used only for baking. In addition, this difference was accentuated by the storage treatment.

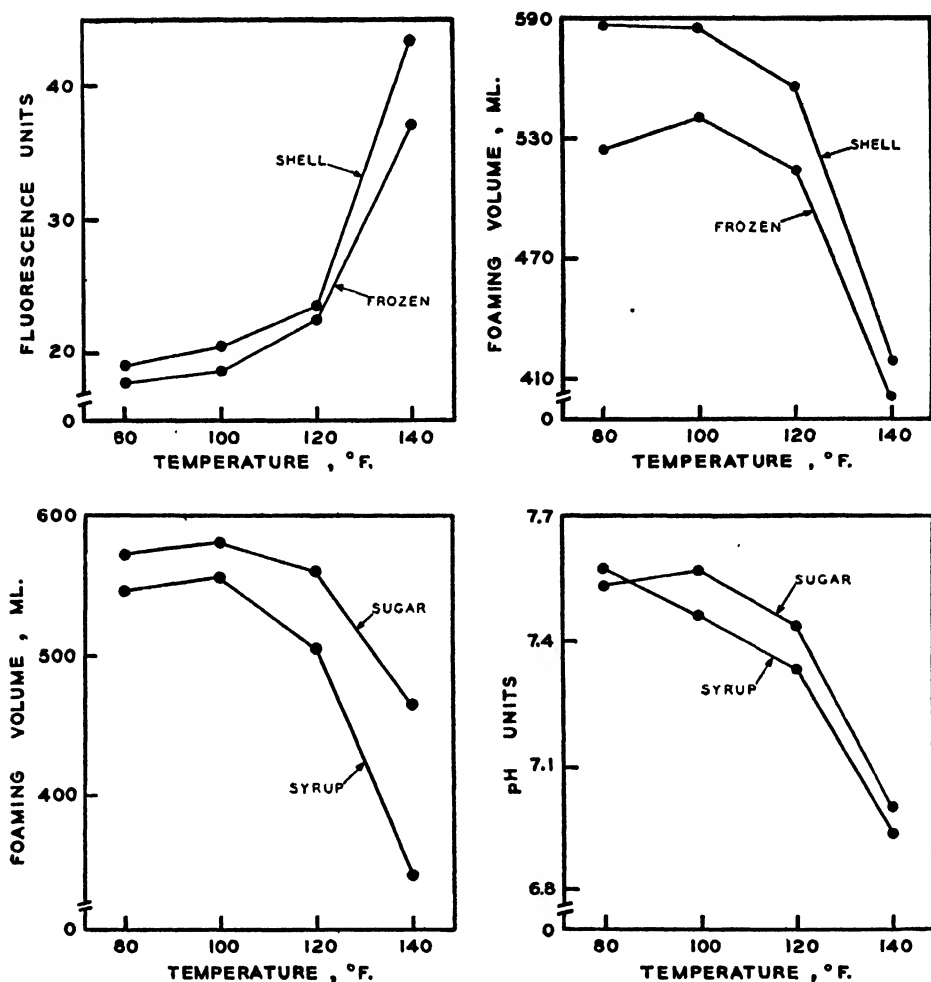


FIG. 4. Average effect of temperature on some of the quality measurements applied to sugar-egg powder prepared from shell and frozen eggs, using sucrose crystals and sucrose syrup. Actual moisture content of these samples was about 2.3%.

The effect of temperature on the fluorescence and foaming volume measurements of sugar-egg powders made from shell egg and from frozen melange, is presented graphically in Fig. 4. Powder produced from frozen melange had lower fluorescence values at all storage temperatures studied. At 80°, 100°, and 120°F. increases in fluorescence were slight and approximately parallel, while at 140°F. both showed very rapid increases in fluorescence development. The divergence of the curves indicated that powder from frozen melange developed fluorescing substances more slowly as the storage temperature was increased from 120° to 140°F. The foaming volume curves showed the pronounced superiority of shell eggs over frozen melange as a

component of sugar-egg powder when subjected to storage temperatures of 80°, 100°, and 120° F.

Sugar-egg powder prepared with syrup and stored at elevated temperatures was more susceptible to deterioration than the product made with granulated sugar (Fig. 4). Although the foaming volume of the latter product, after storage at 80° and 100° F. was better by only about 35 ml., this difference progressively increased to approximately 125 ml. at 140° F. Since a decrease in pH also accompanies quality deterioration it is evident from the pH curves that egg powder prepared with syrup deteriorated more rapidly than that prepared with sugar, when the powders were stored at 100° and 120° F. At 80° and 140° F. the difference between mean pH values, although favouring the powder prepared with sugar, was very small.

Discussion

The results of the heat treatment study show that the addition of sugar to egg prior to drying helps to maintain those qualities desirable for baking. Cooling after drying was less important for sugar-egg powder than it was for plain egg. Nevertheless, it is believed desirable to maintain the cooling practices in current use by industry.

The results of the moisture study indicated that the water content of sugar-egg powder should be below 2.8% and preferably about 1.4% if quality comparable to that of fresh egg powder is to be maintained during storage.

For comparison of the effect of moisture content in sugar-egg powder there was no need to correct fluorescence and potassium chloride values for the presence of added sugar, since all measures were relative. However, to compare plain and sugar-egg powders some adjustment was necessary. This has been done for the potassium chloride values shown in Fig. 5. Since the increase in fluorescence due to the caramelization of the sugar was difficult to evaluate, no correction was made for the fluorescence values of sugar-egg powder. However, it was believed that even if this correction were made the fluorescence curves in this figure would be only slightly altered and the conclusions would be much the same.

Since the sugar in the dried product can be assumed to have a negligible moisture content, all the moisture in sugar-egg powder is probably in the egg fraction. It is possible that the moisture may be equally distributed throughout both sugar and egg fractions, and the beneficial effects attributable to sugar may be due to the ability to dry to a low moisture content, thereby reducing the moisture in the egg fraction. The solid circles and squares shown in Fig. 5 represent an interpolated value for plain egg powder at 3.5% moisture and 120° F. from data previously reported (14) and an actual value for plain egg powder at 3.9% moisture and 120° F. from the heat treatment study, determined after two days' storage. The fluorescence increment and potassium chloride decrement of sugar-egg powders indicated that this product when stored at 120° F. changed by about the same amount as plain egg

powder stored at 110° F. for the same time and certainly much less than can be expected for plain egg powder stored at 120° F. This comparison indicates that sugar exerts a pronounced beneficial effect separate from any suggestion of low moisture content in the egg fraction attributable to distribution of the moisture between the egg solids and the sugar solids.

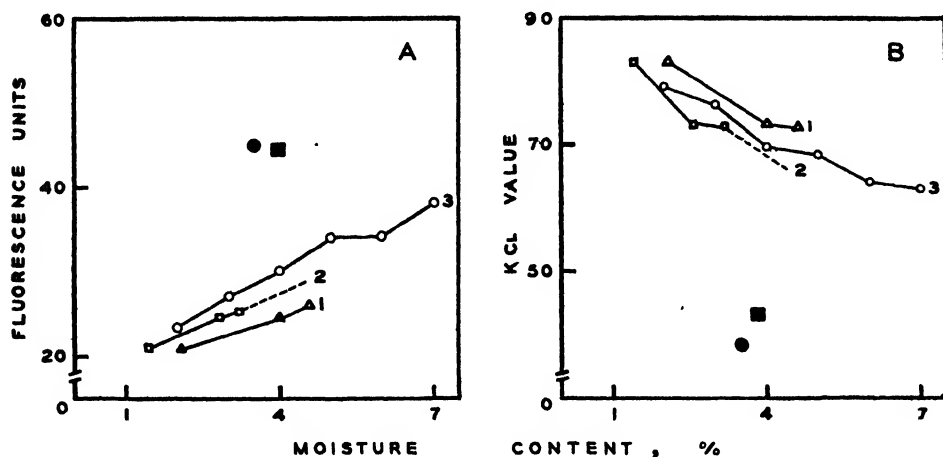


FIG. 5. Effect of moisture content and added sugar on the fluorescence and potassium chloride values of dried egg powder after two days' storage. Curve 1—sugar-egg stored at 120° F.; moisture content calculated on basis of egg solids. Curve 2—sugar-egg (actual moisture content) stored at 120° F. Curve 3—plain egg powder (actual moisture content) stored at 110° F. (15). ■ Plain egg powder, 3.9% moisture, from heat treatment study, storage temperature 120° F. ● Plain egg powder, 3.5% moisture, value interpolated for 120° F. from data previously reported (14).

Some physical or chemical combination may occur between sugar and the components of the egg, and provide protection to the product not only during the drying process but during subsequent handling. Although the nature of this combination is at present in doubt, the practical aspects are of significance, and are receiving further attention in these laboratories.

The results of the study on powder prepared in different ways showed that the storage life of sugar-egg powder was improved by adding granulated sugar instead of syrup to the liquid egg prior to drying. This may be explained on the basis of drying operations. The mixture made with syrup had a higher moisture content than the mixture prepared with solid sugar. Therefore, to obtain the same production rate in terms of solids requires a higher drying temperature (4, 12, 17), and corresponding deterioration in the product would be expected. To obtain drying at the same inlet and outlet temperatures necessitates reduction in melange input. If it is assumed that the liquid particles from the egg-syrup mixture are identical in size with the liquid particles from egg-sugar mixture, the dried particles would be smaller, settle more slowly and, as a result, may be exposed to a longer period of heating in the drier, thus causing reduction in quality of the product.

This study also showed that melange from fresh shell eggs produces a sugar-egg powder superior to that from frozen melange. Egg melange is a colloidal complex containing in solution proteins, fats, a trace of sugar, lecithin, and about 1% of salts. It is known that colloids when frozen do not normally recover their original state on thawing, a phenomenon that is probably due to precipitation or coagulation of the proteins during freezing. It has been observed that egg yolk, frozen, stored below -6°C . for a reasonable time and then thawed, lost its fluidity and passed into a viscous condition with a reduced volume (6). A similar treatment caused the white to separate into liquid and viscous parts with the former increasing at the expense of the latter by an amount depending upon the temperature and storage time. The rate of freezing and thawing of the egg has also been found to affect the composition of the resulting melange (6). These changes may be responsible, in part at least, for the differences in behaviour of powders prepared from shell egg and from frozen melange.

Acknowledgments

The authors wish to express their appreciation of the assistance rendered by Mrs. Margaret Reid, Biochemist, and by Mr. D. H. Whitteker, Laboratory Assistant.

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LIQUID AND FROZEN EGG

II. METHODS OF DETERMINING SOLIDS CONTENT OF LIQUID AND FROZEN EGG¹

BY C. G. LAVERS² AND JESSE A. PEARCE³

Abstract

Solids content of liquid egg prepared from shell eggs having different histories was measured by the official A.O.A.C. vacuum oven method and compared with measurements of specific gravity and refractive index. Specific gravity measurements were the least satisfactory, but may provide a rough check on solids content. Refractive index measurements following treatment with ammonium hydroxide were a more satisfactory measure of solids content than the same measurement on liquid egg after treatment with trypsin solution.

Relations between the solids content of defrosted frozen egg and unfrozen liquid egg and refractive index, as determined with a Zeiss sugar refractometer and with a hand sugar refractometer, were calculated for the method involving the addition of ammonium hydroxide. The relation, solids-refractive-index, for unfrozen liquid egg differed from the relation for frozen egg. However, the method provided a rapid, convenient, and accurate means for determining solids content.

Introduction

In recent years dried egg production has increased markedly. Since drying capacity is limited, all the eggs produced during the peak laying season cannot be dried or used immediately. Much of this egg is frozen and held in storage for subsequent drying, or for use as thawed liquid egg by bakers and others. One problem of importance in drying and freezing liquid egg is that of a rapid test for the solids content. The use of refractive indices for this purpose has been described (4). The present paper compares two of these refractometric methods (2, 4) and a hydrometric method with the official A.O.A.C. vacuum oven method (1, p. 308).

Materials and Methods

Preliminary experiments indicated that liquids prepared from eggs having different histories differed in their initial solids content, and in their behaviour on dilution. Therefore, dilutions of liquid prepared from eggs with a variety of histories were used in this study. To compare the various methods, the following eggs were used: currently available Grade A large summer eggs; Grade A large summer eggs obtained within one day of laying; Grade A large spring eggs, stored at 0° C. (32° F.) for three months (all grades refer to condition of eggs before storage); Grade A large spring eggs held in commercial storage for three months; Grade A large spring eggs stored at 0° C. for three months followed by two weeks at 21.1° C. (70° F.); currently available

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Grade A pullet summer eggs; and Grade C summer eggs. To obtain a more representative sample, eggs of the following grades were added to the above in determining the final relation between refractive index and solids content; Grade A large early spring eggs; Grade A pullet spring eggs; Grade C spring eggs. In addition, frozen egg of five different grades, ranging from top quality to mouldy, was used in the investigation.

All samples of both liquid and defrosted frozen egg were prepared for analysis by mixing with a "Mixmaster" at low speed. The official A.O.A.C. vacuum oven method for determining solids content (1, p. 308) was used as a standard against which the various methods were compared.

Specific gravities were determined using a hydrometer having a range of 1.000 to 1.070 (60°/60° F.). In making the determination, it was found necessary first to remove the foam from the surface of the liquid egg, allow the hydrometer to sink gently in the liquid to a steady position, then push it down one or two scale divisions and let it rise to an equilibrium position. Through a series of 35 determinations at temperatures ranging from 10° to 35° C. (50° to 95° F.) the temperature gradient was observed to be -0.00032 specific gravity units per °C. rise in temperature (-0.00018 units per °F.). Using this figure, all determinations were corrected to give specific gravity at 15.6° C. (60° F.).

The two refractometric methods used have been described (2, 4). One of these depended on an enzymatic (trypsin) digestion (4), the other on the use of an electrolyte, 28 to 29% reagent grade ammonium hydroxide (2). The former method involved the use of a trypsin solution (trypsin, 500 gm.; water, 770 ml.; and 0.25 *N* sodium hydroxide, 800 ml.), having a refractive index of 1.377 at 30° C. To 10 gm. of whole egg, 1.8 ml. of this solution was added, followed by thorough mixing. Several drops of the mixture were placed on the prisms of the refractometer and after 30 sec. the refractive index was read. The latter method required the addition of 10 drops of ammonium hydroxide to 20 ml. of the egg sample, mixing, and determination of the refractive index as before. All refractive indices were determined at 30° C. $\pm 0.5^\circ$ C.

For the major portion of this work a Zeiss sugar refractometer was used. In addition, the use of a hand refractometer was considered as a more convenient method for plant purposes. This instrument was a Bausch and Lomb hand sugar refractometer, reading from 0 to 60% sugar.

Results

Comparison of Methods

Refractometric and hydrometric measurements showed a high correlation with solids content as determined by the official A.O.A.C. vacuum oven method. Equations expressing the relation between these rapid determinations and per cent solids in liquid egg, and estimations of the error that would be involved in using them to predict solids content, are given in Table I. While the equations relating solids content in any one type of egg to the other measurements differed somewhat in slope (Figs. 1, 2, and 3), these differences

TABLE I

EQUATIONS, WITH ERRORS OF PREDICTION, RELATING PER CENT SOLIDS IN LIQUID EGG (y) TO VALUES BY RAPID METHODS (x)

Method	Equation	Error of prediction, % solids
Specific gravity (hydrometric)	$y = 793.02 x - 794.77$	± 1.3
Refractive index (enzymatic)	$y = 678.04 x - 907.94$	± 0.66
Refractive index (electrolytic)	$y = 574.75 x - 766.02$	± 0.42

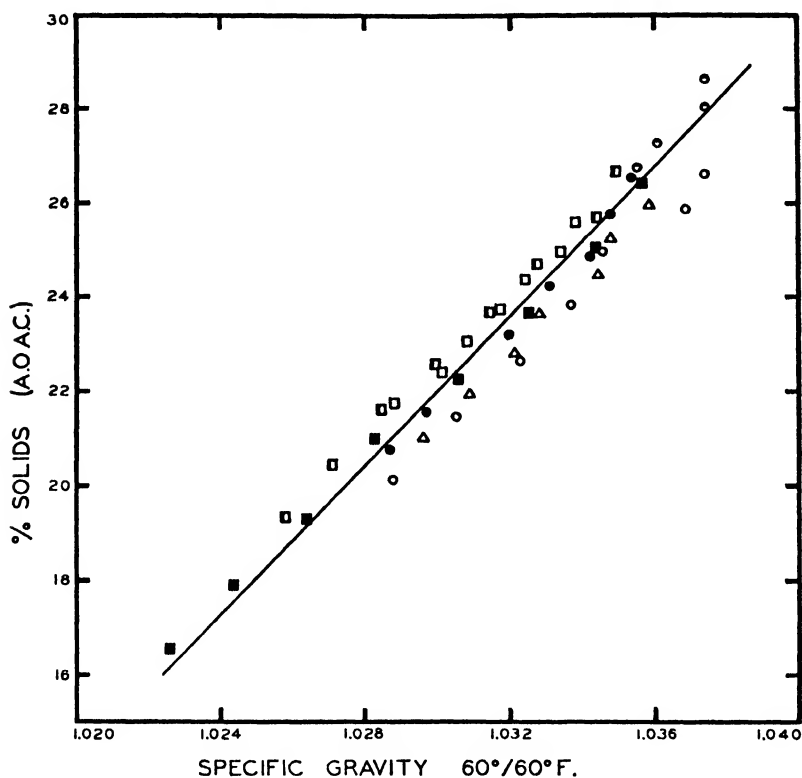


FIG. 1. Relation between solids content of liquid egg and specific gravity.

Key

- Grade A large summer eggs obtained within one day of laying.
- Currently available Grade A large summer eggs.
- ▣ Currently available Grade A pullet summer eggs.
- Grade A large spring eggs held three months at 0° C. (32° F.).
- Grade A large spring eggs held three months in commercial storage.
- ◐ Grade A large spring eggs held three months at 0° C., then two weeks at 21.1° C. (70.0° F.).
- △ Grade C summer eggs.

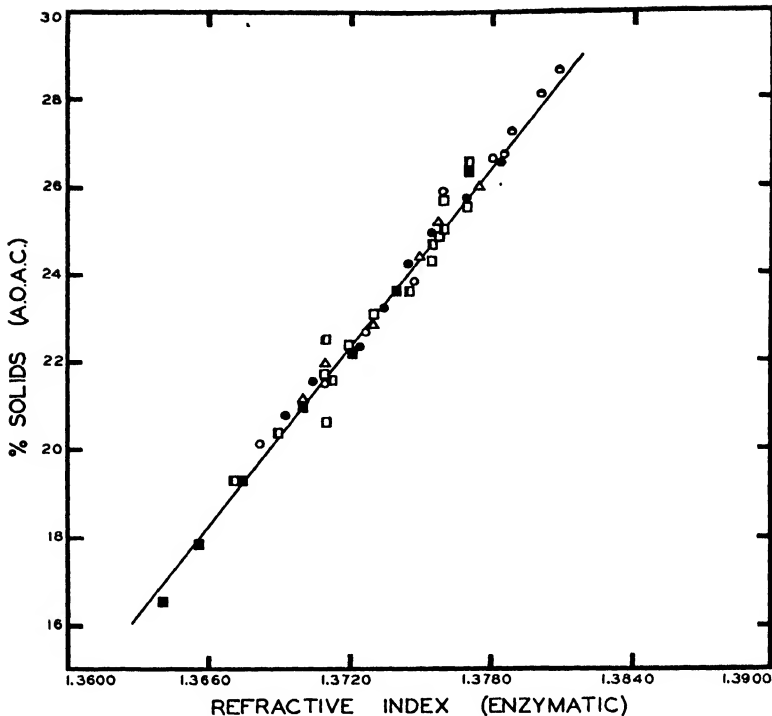


FIG. 2. Relation between solids content of liquid egg and refractive index after treatment with trypsin (Key in caption for Fig. 1.).

did not hinder combination of the data to establish the mean relations shown in the figures. These equations are based on equal numbers of determinations performed on identical samples, and are of value chiefly for comparative purposes. Best estimate equations recommended for predicting solids content from refractive index (electrolytic method) are discussed later.

The error in determining solids content by the specific gravity method was of sufficient magnitude to preclude its use, except as a rough check. It was evident that differences resulting from previous history exerted a pronounced influence on the relation obtained. This method was unsatisfactory for frozen egg owing to the lack of homogeneity in the thawed material. Attempts at homogenizing the melted egg resulted in the incorporation of air into the product, which did not pass off even on long standing, thereby preventing accurate determinations.

Both refractometric methods were relatively satisfactory (Table I and Figs. 2 and 3). Refractive index measurements on liquid egg after treatment with ammonium hydroxide was the most satisfactory of the methods investigated. The accuracy of prediction was somewhat better. The use of ammonium hydroxide rather than the trypsin solution was simpler from the

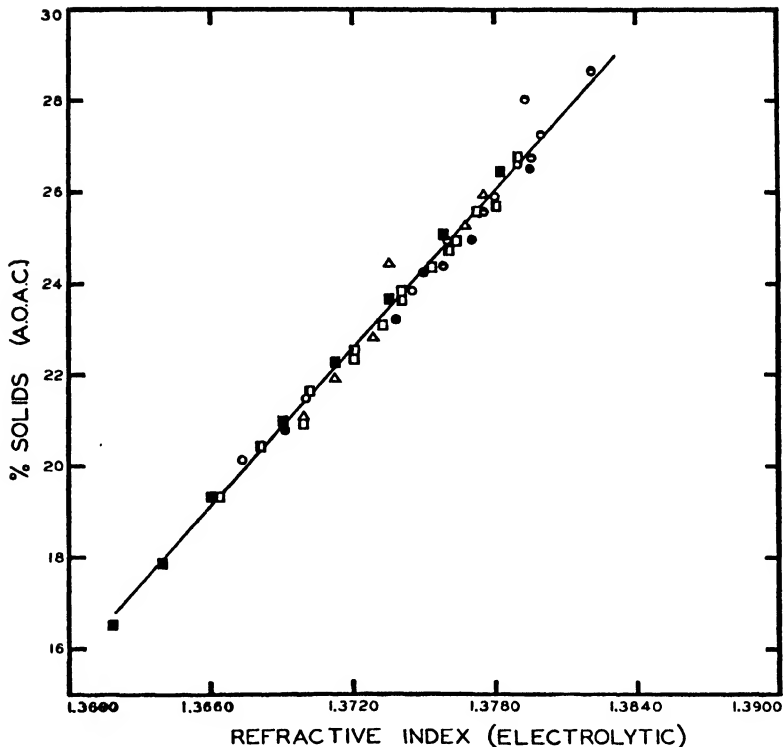


FIG. 3. Relation between solids content of liquid egg and refractive index after treatment with ammonium hydroxide. (Key in caption for Fig. 1.)

point of view of laboratory technique, and in addition gave a more easily readable field in the refractometer.

Before putting any of the above determinations into practical use as a standard method of measuring solids in liquid egg, it would be advisable to evaluate differences of technique existing between laboratories. The calibration curves shown in Figs. 2 and 3 are approximately 0.5 and 1% lower respectively than those previously recorded (2, 4). This disagreement may be attributable to the different methods of obtaining liquid egg of varying solids content.

Best Estimate Equations

Since the initial comparison showed the refractometric method involving the use of ammonium hydroxide to be the best of those considered, it was used in all subsequent work. Further measurements were made using this method to include determinations on eggs produced over the major portion of the Canadian laying season in computing the final prediction equation. The method was also used on thawed frozen egg. Equations, and errors of prediction, for both liquid and melted frozen egg are given in Table II. These relations are shown graphically in Fig. 4. It will be noted that the equations

TABLE II

EQUATIONS, WITH ERRORS OF PREDICTION, RELATING PER CENT SOLIDS IN EGG (y) TO REFRACTIVE INDEX, ELECTROLYTIC METHOD (x)

Kind of egg	Equation	Error of prediction, % solids
Liquid	$y = 593.53 x - 791.68$	± 0.76
Frozen	$y = 587.08 x - 781.56$	± 0.48

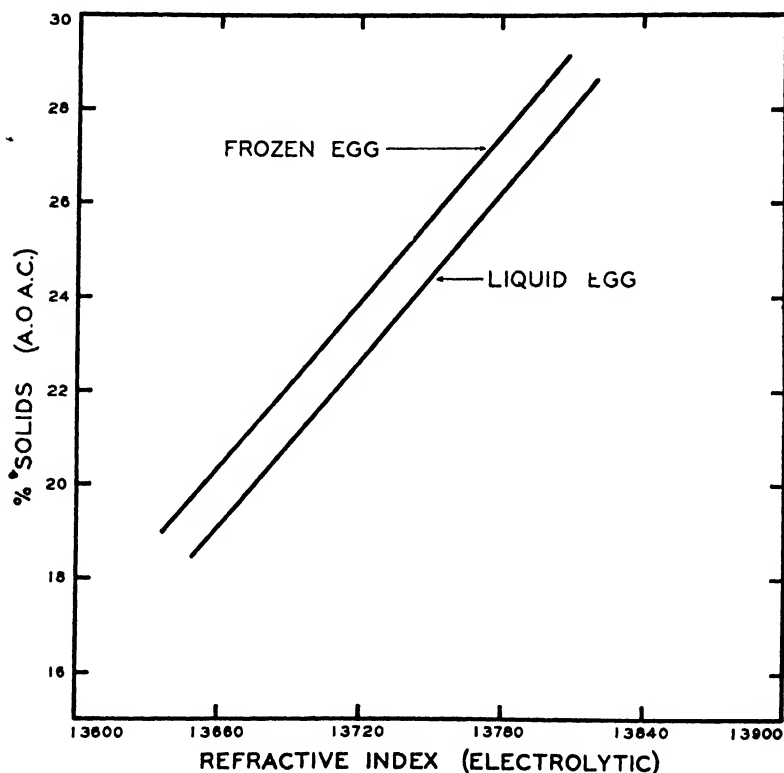


FIG. 4. Relations between solids content of egg (liquid and frozen) and refractive index after treatment with ammonium hydroxide (as calculated from the best estimate equations).

for liquid and frozen egg have approximately the same slope, but quite different intercepts, and so are not interchangeable.

This method of determining solids in egg is rapid, simple, and sufficiently accurate for industrial purposes.

Determinations Using a Hand Refractometer

The instrument used in the refractometric work so far described was not portable, and required a constant temperature bath. For routine inspection

a portable instrument would be very advantageous. For this reason, consideration was given to an easily obtainable, relatively inexpensive hand refractometer, designed to determine sugar concentration directly. No method of temperature control was provided for this hand instrument, but a correction thermometer reading in per cent solids for sugar was mounted on the side of the refractometer.

For a series of 18 readings at temperatures ranging from 19.0° to 32.0° C. (66.2° to 89.6° F.) the change of refractive index of liquid egg with temperature was observed to be -0.00012 refractive index units per °C. rise in temperature, (-0.00007 units per °F.). The corresponding figure for sugar solutions, as calculated from available data (3), is approximately -0.00014 (-0.00008). Since these two figures are nearly the same, the temperature correction given for sugar may be applied directly to egg.

Since the relation between refractive index and per cent sugar of sugar solutions is known (3), and that between refractive index (electrolytic method) and per cent egg solids is given in Table II, it was possible to calculate the equations given for converting readings on the hand sugar refractometer to per cent egg solids (Table III). Although these equations could be calculated it was necessary to know the error of prediction involved when using this hand instrument. To evaluate this, a series of 23 determinations was made on identical samples using both the hand and Zeiss sugar refractometers. This gave a direct comparison of the error involved in using each instrument. With this knowledge it was possible to calculate, by simple ratio, the error of prediction involved when using the hand instrument and the prediction equations given in Table III, because these equations were based on the same determinations as those given in Table II.

TABLE III

EQUATIONS, WITH ERRORS OF PREDICTION, RELATING PER CENT SOLIDS IN EGG (y) TO READINGS ON A HAND REFRACTOMETER (x)

Kind of egg	Equation	Error of prediction, % solids
Liquid	$y = 1.024 x - 3.59$	± 0.56
Frozen	$y = 1.016 x - 2.14$	± 0.35

The procedure for determining per cent solids with the hand refractometer would then be as follows: Prepare the sample and place on the prism as previously described; read the instrument (% sugar); read the temperature correction thermometer and apply the correction to the figure just read; convert the corrected figure to per cent egg solids using the equations given in Table III. This instrument, combined with the electrolytic treatment of the egg, provides a rapid, convenient, and inexpensive method for determining the solids content of egg in industrial practice.

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A SURVEY CAMERA TO FIT A BERGER TRANSIT¹

BY R. A. NODWELL² AND R. C. BURSTOW³

Abstract

An accurate survey camera covering a horizontal angle greater than 45° on $3\frac{1}{4}$ by $4\frac{1}{4}$ in. plate or film is described. The camera is easily interchanged with the telescope of a Berger transit.

A small, accurate survey camera, which was designed and constructed at the request of the Department of Lands, Forest Branch, Province of British Columbia, is described in this paper. The camera takes $3\frac{1}{4}$ by $4\frac{1}{4}$ in. plate or film, covers a horizontal angle of approximately 50° , is interchangeable with the telescope of a Berger transit, and is light and compact for ease in transport.

Frequently lenses of very small f -numbers have been used in cameras of this type. Recent tests on lens-film resolving power of photographic lenses have indicated that best average photographic resolving power is obtained at medium apertures of $f/11$ or $f/16$. With further increase in aperture the aberrations become large and with decrease in aperture the loss in resolving power due to diffraction of light greatly exceeds the gain due to decrease in aberrations. This would indicate that the best aperture for the camera would be about $f/11$. However, a Ross 4 in. $f/4$ wide angle Xpres survey lens, which could be stopped down to $f/16$, was available, and, since a wider aperture might be useful at times in spite of decrease in resolving power, it was decided to equip the camera with this lens. The lens is mounted in a Compur shutter with a speed range from "Time" to "1/200 sec."

Since any stray light that falls on the photographic plate reduces the contrast and resolving power, it is very important that adequate baffling be provided inside the camera body. A relatively large camera cone is required to accommodate this baffling. This makes it necessary to mount the camera with the lens located approximately between the uprights of the transit. Thus the centre of gravity of the camera is not over the axis of rotation, but it was found that the resulting stress did not impair the accuracy of the transit.

The accurate location of the photographic plate in the focal plane is accomplished in the following manner. The camera back, *A*, (Fig. 2), which retains the plate or film holder, is free to move about $1/16$ in. in a direction parallel to the axis of the lens. The movement of this back is controlled by two cams, *B*, which are rotated by the handle, *C*. The camera back is pulled against the cams by two internal leaf springs. Thus with the back, *A*, in its forward position the plate or film is held in direct contact with the surfaces, *D*, of the

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camera body. These contact surfaces define the focal plane of the camera and hence the principal distance is independent of any dimension of the plate holder.

The principal point is defined by four fiducial marks, *E*. When the transit is level the principal axis is held horizontal by means of the screw, *F*, (Fig. 1), and an opposing screw that bears on a hardened steel bar mounted on the transit. The opposing screw is firmly held by a lock nut, which may be loosened in order to make small adjustments on the screw when the instrument is being checked in the field. The fiducial marks are so adjusted that the line joining the transverse marks is horizontal and that joining the other two is vertical.

The drum, *G*, which casts a silhouette of a number from 1 to 8 onto the plate or film, is rotated by an exterior wheel, *H*. This wheel displays a number corresponding to that being recorded on the film or plate.

The camera is equipped with three interchangeable filters. A ground glass in a suitable holder is also supplied.

Fig. 3 shows the camera and its accessories in the case.

Acknowledgment

The authors wish to express their appreciation to the members of the Optics and Metrology Sections who assisted in the construction and calibration of this camera.

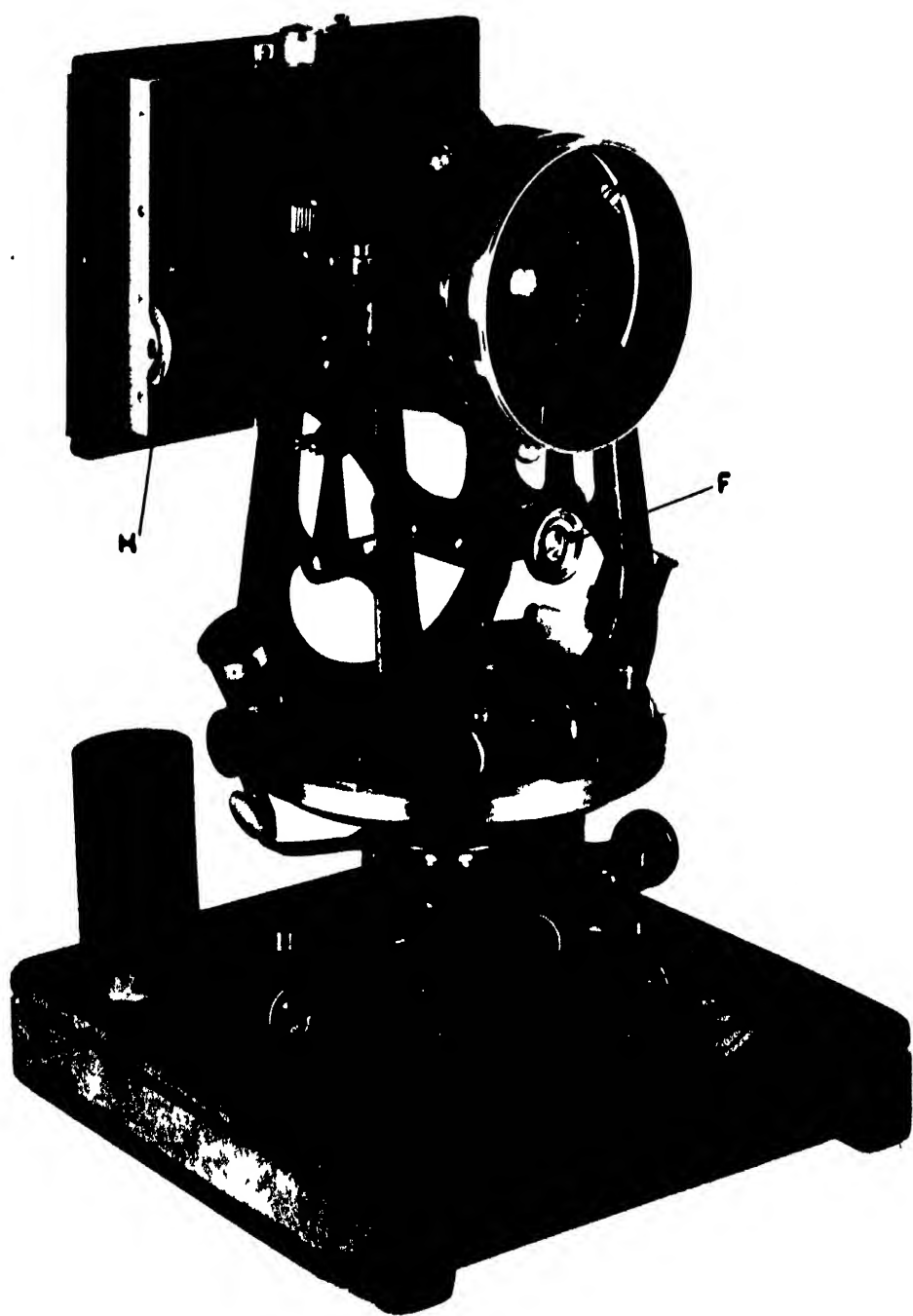


FIG. 1. *The camera mounted in the transit.*

PLATE II

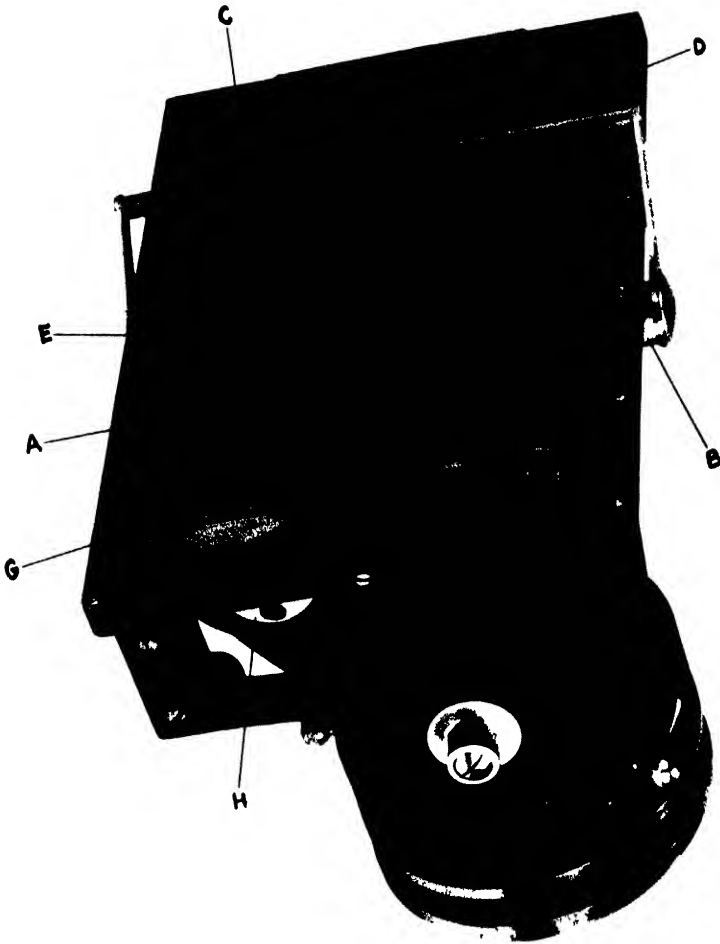


FIG. 2 View showing back of camera.

PLATE III

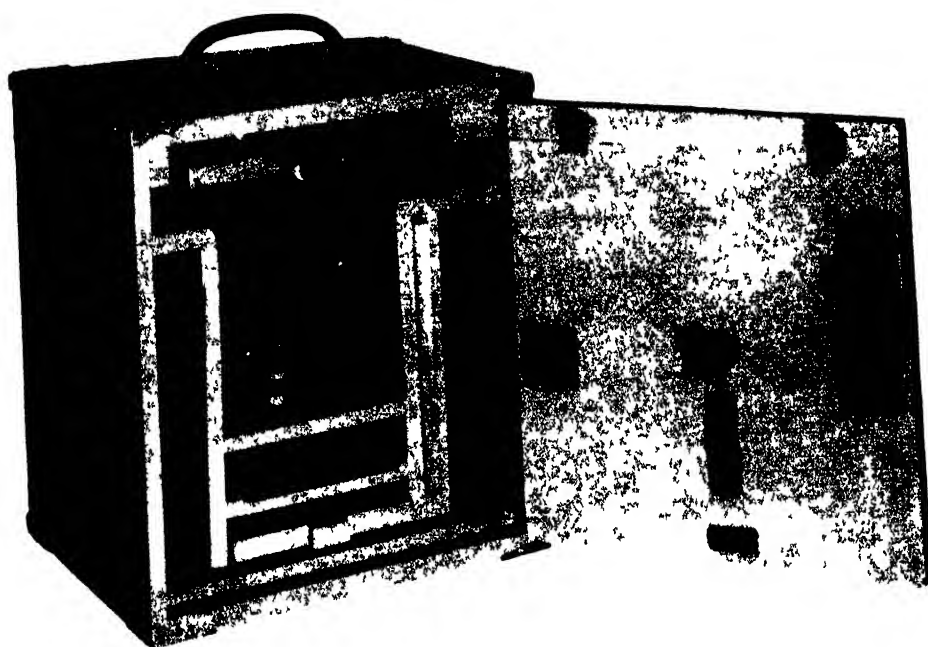


FIG. 3 The camera and its accessories in the case

THE EFFECT OF WEATHERING ON COTTON FABRIC CONTAINING CERTAIN COPPER ROTPROOFERS¹

C. H. BAYLEY² AND M. W. WEATHERBURN²

Abstract

On exposure to outdoor weathering for three months, No. 8 cotton duck showed substantial loss in breaking strength. The untreated fabric showed a loss of the same order as the losses of samples treated with copper naphthenate, copper hydroxynaphthenate, copper oleate, and copper tallate containing 0.1 to 1.0% copper. The copper treated samples showed slight evidence of increased actinic degradation as measured by cuprammonium fluidity. There was an appreciable decrease in the copper content of the treated samples on weathering. The decrease in copper content and breaking strength on weathering and the extent of attack by micro-organisms in soil burial testing were reduced considerably by the presence of a waterproofing compound of the wax-pigment-filler type. The initial water resistance of the proofing was modified by the presence of the copper compounds, being reduced by copper naphthenate, oleate, and tallate and increased by copper hydroxynaphthenate although on ageing and weathering these effects were minimized.

During the past four years considerable use has been made in the United States and Canada of organic compounds of copper as rotproofers for cotton fabrics. The more commonly used of these compounds include copper naphthenate, hydroxynaphthenate, oleate, and "tallate". Copper "tallate" is a mixture of copper derivatives of the organic acids present in "tall oil",* a product of the processing of southern pine (6).

It is well known that cellulose materials such as cotton duck used in tents, tarpaulins, etc., undergo actinic deterioration when exposed to sunlight for extended periods. It is also known that the presence of certain added constituents has an important effect on this actinic degradation. Whittaker (7) has studied the tendering effects of certain types of dyestuffs on cotton and rayon. On the other hand, chromium oxide precipitated on cotton cloth appears to reduce actinic degradation (4). Fargher has reported (5) that the danger of accelerated actinic degradation resulting from the presence of copper is small, and is more likely to occur in heavy, closely woven cloths than in thin open ones, probably owing to the slower rate of loss of copper from the former.

In view of the wide use of copper soaps as rotproofers it was of interest to determine the effect of such compounds on the actinic degradation of cotton fabric. The processors in Canada of tarpaulins for Service transport vehicles have utilized a waterproofing compound of the wax-pigment-filler type containing copper naphthenate applied to No. 8 cotton duck. Therefore, in this study use was made of copper naphthenate and other copper soaps applied

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* The acids present in tall oil consist of fatty and resin acids, chiefly oleic, linoleic, linolenic, and abietic.

to No. 8 cotton duck both with and without the addition of waterproofing materials.

Samples containing a range of copper contents were prepared and the effect of exposure to weather on breaking strength, cuprammonium fluidity, water resistance, and rot resistance determined.

Materials Used

The copper compounds used were commercial preparations of the naphthenate, hydroxynaphthenate, oleate, and tallate*.

The fabric used was unbleached No. 8 duck, all the samples being taken from the same length of fabric. The treatment of the samples was carried out on specimens 9 by 36 in. in the case of the laboratory samples, 180 by 36 in. in the case of the commercial samples. The samples containing the copper compounds alone were prepared in the laboratory as previously described (1). In the case of copper hydroxynaphthenate, which did not dissolve completely in Stoddard solvent, the treating mixture contained an appreciable amount of a pale blue precipitate. The samples carrying the pigmented proofing treatment in addition to the copper compounds were prepared by the Dominion Oilcloth and Linoleum Co. The colour of the latter samples, purple-brown, was obtained by the use of an iron oxide pigment. The proofing mixture containing the copper compound was applied in paste form by spreading. In proofings of this type the amount of material added to the fabric is approximately 40% on the weight of the original fabric, about one-half of this being wax. The proofing is one that completely seals the fabric, and the wax may be regarded as extending in a continuous film throughout the fabric.

The copper contents aimed at were 0.1, 0.3, 0.5, and 1.0%. These were readily obtained in the case of the laboratory samples but in the case of the commercial proofings the samples containing the higher percentages of copper showed somewhat lower amounts of copper than were desired. However, it was believed that the copper contents in the two series were sufficiently similar to permit a comparison of the results.

In this paper the term "treated" refers to the presence of various copper compounds alone, while the terms "proofed" or "unproofed" refer to the presence or absence of the waterproofing wax-pigment-filler mixture.

Test Methods

The weather exposures were carried out on the roof of the National Research Laboratories, Ottawa, from July 10 to October 10, 1944, the samples being attached to wooden racks and slung at an angle of 45°, facing southwest. Each exposure specimen consisted of duplicate samples sewn together with a double seam in the long dimension. The specimens were protected from

* While considerable use has been made in Australia of copper oleo-stearate as a rotproofing, this material has not been used to any appreciable extent on this continent, and was not investigated.

contact with the wooden frames by means of 16 by 10 in. runners of unbleached duck sewn across the ends of the specimens, and were attached to the frames by cotton ropes fastened to grommets situated at the corners of the runners.

The methods of test used were those given in Schedule 4-GP-2-1944 of the Canadian Government Purchasing Standards Committee. Breaking strengths were determined by the 1 in. ravelled strip method, those on the controls being carried out on samples that had been leached in water to compensate for shrinkage resulting from wetting during weathering. Rot resistance was determined by the soil burial method, tests being carried out on: (a) samples that had been subjected to weathering, (b) samples that had not been weathered, (c) samples that had not been weathered but had been subjected to a period of leaching. The leaching treatment involved exposure of each sample (6 by 15 in.) in a bottle of 1 litre capacity to the action of water at 25° C. flowing at a rate of 10 litres per hour for 24 hr. The water resistance of the proofed samples was determined by the variable-head method.

Copper determinations were carried out by the ignition method (3). Determinations of cuprammonium fluidity were performed on samples from which the solvent-soluble portion of the copper compounds had been removed by extraction with Stoddard solvent, this measurement being made on the unproofed samples only since it was not possible to completely remove all the constituents of the waterproofing compound.

Data

(1) Weather Conditions

The data for weather conditions during the exposure period are given in Table I. It will be noted that the weather was fairly warm, with considerable sunshine, and not much rain, particularly in July and August. The conditions

TABLE I
RECORD OF WEATHER CONDITIONS DURING THE EXPOSURE PERIOD

Period	Mean daily temp., °F.		Rainfall, in.	Sunshine, total hr.
	Maximum	Minimum		
July 10 - July 16	82	59	0.53	56.6
July 17 - July 23	78	52	1.09	69.8
July 24 - July 30	81	62	0.74	45.8
July 31 - Aug. 6	88	63	0	77.6
Aug. 7 - Aug. 13	90	57	0	80.0
Aug. 14 - Aug. 20	85	59	0.25	62.9
Aug. 21 - Aug. 27	76	51	0.20	61.8
Aug. 28 - Sept. 3	77	56	0.60	45.4
Sept. 4 - Sept. 10	70	50	0.33	39.6
Sept. 11 - Sept. 17	78	55	0.74	32.6
Sept. 18 - Sept. 24	69	49	0.87	37.9
Sept. 25 - Oct. 1	55	43	1.80	32.3
Oct. 2 - Oct. 10	60	41	0.58	33.1
Average	76	54	Total 7.73	675.4

were, therefore, such as to favour actinic degradation. However, there was some evidence of superficial fungus growth on the underside of the untreated control sample of unbleached duck. The organism noted was a species of *Alternaria*.

(2) *Breaking Strength Loss*

The data are given in Table II, while Table III gives an analysis of variance of the breaking strength losses as given in Table II. It is apparent that the effects of proofing, treatments, and concentrations are highly significant. The following additional observations may be made from a study of the table, bearing in mind the differences specified as necessary (5% level of statistical significance).

(a) *Weathering Effects*

The samples containing copper hydroxynaphthenate showed lower breaking strength losses than did the samples containing the other compounds, this effect being more pronounced in the unproofed samples. On the whole, the losses shown by the samples containing oleate are somewhat greater than in the case of the naphthenate and tallate, particularly in concentration of 1% copper. In general the unproofed samples showed considerably greater losses than the proofed samples. The losses shown by the unproofed samples containing 0.1% copper are lower than those of the unproofed samples containing higher percentages of copper. This effect is not observed in the proofed samples, there being no indication that the losses are proportional to the copper content. Comparing the unproofed and proofed samples containing the various compounds with the untreated controls, it will be seen that there is no evidence that the presence of copper resulted in any marked increase in breaking strength losses, except possibly in the case of the sample containing 1.13% copper in the form of copper oleate (Sample OL 4).

(b) *Burial Effects*

When subjected to exposure followed by soil burial, the breaking strength losses shown by the proofed samples are considerably lower than those of the unproofed samples for all concentrations of copper and for all compounds investigated. In the case of the unproofed samples, the losses shown by those containing hydroxynaphthenate and tallate are similar, and are lower than those shown by the naphthenate and oleate, which are also similar.

When subjected to soil burial without previous exposure, the breaking strength losses of the unproofed samples containing copper naphthenate are considerably lower than those containing the other compounds, the latter being similar to each other. The proofed samples show losses similar to those of the unproofed, except in the case of the samples containing copper hydroxynaphthenate in concentrations of 0.1 and 0.5% copper, and copper oleate and tallate containing 0.1% copper, in which the losses of the proofed samples are lower. With regard to the effect of the copper content of the samples, the losses shown by samples containing 0.1% copper are considerably greater than those containing higher concentrations of the metal, the only exception

TABLE II
BREAKING STRENGTH LOSSES

Sample	Original copper concentration, %	Breaking strength loss, %, after			
		Exposure 3 months	Exposure and burial 2 weeks	Burial 2 weeks (unleached, unexposed)	Burial 2 weeks (leached, unexposed)
<i>A. Not proofed</i>					
Untreated UN	0	36.9	73.2	96.5	—
Copper naphthenate					
N1	0.096	18.7	36.9	0.8	45.2
N2	0.31	34.5	39.6	+10.4	44.2
N3	0.48	43.3	44.5	1.2	47.8
N4	0.98	37.7	43.2	0.8	18.9
Copper hydroxynaphthenate					
HN1	0.13	10.8	23.7	37.3	80.9
HN2	0.31	18.3	25.5	21.5	45.8
HN3	0.64	30.2	26.3	9.4	52.9
HN4	1.10	26.6	33.6	12.5	46.9
Copper oleate					
OL1	0.090	28.0	37.2	36.5	64.4
OL2	0.32	44.0	46.8	8.6	35.6
OL3	0.58	43.0	44.5	11.0	22.8
OL4	1.13	53.3	52.2	4.7	1.2
Copper tallate					
T1	0.072	27.7	22.1	37.8	49.4
T2	0.34	37.5	33.3	18.0	42.6
T3	0.54	46.4	39.2	3.1	38.7
T4	1.08	36.4	35.3	2.2	10.1
<i>B. Proofed</i>					
Untreated PUN	0	24.5	69.8	94.9	93.0
Copper naphthenate					
PN1	0.093	24.5	18.1	2.3	6.8
PN2	0.21	12.1	9.8	+ 4.5	+ 6.9
PN3	0.37	18.4	18.8	10.2	5.6
PN4	0.62	22.2	18.6	+11.7	+ 2.8
Copper hydroxynaphthenate					
PHN1	0.077	17.8	15.9	20.0	14.8
PHN2	0.17	13.5	15.8	3.1	+ 1.9
PHN3	0.32	13.1	13.8	+ 3.0	4.5
PHN4	0.74	18.6	9.9	+ 4.9	5.3
Copper oleate					
POL1	0.074	18.1	10.6	18.9	5.5
POL2	0.25	20.0	12.9	7.5	3.5
POL3	0.36	22.2	15.9	11.9	7.9
POL4	0.76	26.4	24.0	7.7	+ 8.7
Copper tallate					
PT1	0.088	13.5	10.0	26.0	19.5
PT2	0.22	20.6	17.6	17.6	11.2
PT3	0.41	27.9	17.9	13.0	1.1
PT4	0.83	17.8	11.2	+ 9.1	+ 6.6

TABLE III

ANALYSIS OF VARIANCE OF BREAKING STRENGTH LOSS

Source of variation	Degrees of freedom	Mean square			
		Exposure 3 months	Exposure and burial 2 weeks	Burial 2 weeks (unleached, unexposed)	Burial 2 weeks (leached, unexposed)
Proofed vs. not proofed	1	1596.13**	3678.68**	253.12*	10738.45**
Treatments	3	248.28**	170.93**	417.70**	328.68*
Concentration	3	187.97**	71.68**	698.22**	1052.53**
Treatment \times concentration	9	27.41	24.96	99.90*	97.72
Proofed \times treatment	3	75.92*	98.04**	111.85*	224.22
Proofed \times concentration	3	119.58*	27.31	72.00	247.19*
Proofed \times treatment \times concentration	9	18.92	9.33	24.73	61.12

** Significant at 1% level.

* Significant at 5% level.

being copper naphthenate, which gave good protection even in 0.1% copper concentration.

In certain instances there was an apparent increase in strength after burial. This might be attributed to variation in the strength of the original fabric or to shrinkage brought about by burial which was greater than that produced by the leaching treatment of the control samples. In any case, these values are not statistically significant.

When subjected to leaching in water followed by soil burial the breaking strength losses shown by the unproofed naphthenate, oleate, and tallate treated samples are similar and lower than those shown by the samples containing the hydroxynaphthenate. The breaking strength losses of the unproofed samples containing 1% copper are lower than those for samples containing 0.3 and 0.5% copper, the latter being similar and lower than the losses of the 0.1% copper samples. With the proofed samples the losses are very much lower, particularly in cases where the losses of the unproofed were appreciable, namely, the hydroxynaphthenate in all concentrations, the others in concentrations below 1.0% copper.

(3) Loss of Copper

Measurements of copper losses resulting from the various test procedures were carried out on the samples originally containing approximately 0.3% of the metal (Table IV). It will be noted that exposure resulted in considerable loss of copper (63 to 81%) from the unproofed samples, and also that the losses were considerably less (5 to 24%) with the proofed samples. With the unproofed samples the losses of copper were increased slightly (73 to 93%) as a result of burial following exposure. With the latter samples the copper losses resulting from burial with preliminary leaching were considerably less

TABLE IV
LOSS OF COPPER

Sample	Original copper conc, %	Copper, %, on fabric after				Copper loss, %, after			
		Exposure 3 months	Exposure and burial 2 weeks	Burial 2 weeks	Leaching and burial 2 weeks	Exposure 3 months	Exposure and burial 2 weeks	Burial 2 weeks	Leaching and burial 2 weeks
N2	0 31	0 058	0 022	0 25	0 17	80 7	93 0	19 4	45 2
HN2	0 31	0 10	0 082	0 23	0 18	67 8	73 5	25 8	41 9
OL2	0 32	0 12	0 087	0 22	0 22	62 5	72 8	31 3	31 3
T2	0 34	0 11	0 082	0 28	0 21	67 7	75 9	17 7	38 2
PN3	0 37	0 28	0 28	0 28	0 26	24 3	24 3	24 3	29 8
PHN3	0 32	0 29	0 30	0 30	0 27	9 4	6 3	6 3	16 6
POL2	0 25	0 20	0 20	0 20	0 20	20 0	20 0	20 0	20 0
PT3	0 41	0 39	0 36	0 36	0 34	4 9	12 2	12 2	17 1

than those resulting from exposure alone. Burial without preliminary leaching gave similar losses for both the unproofed and proofed samples, except for the sample containing copper hydroxynaphthenate, but, with samples leached before burial, the presence of the wax proofing decreased the extent of copper loss resulting from burial.

(4) Water Resistance

The measurements of water resistance of the proofed samples were carried out a few days after the application of the proofing treatments, again at the end of the three month exposure and also on a set of unexposed samples that had been stored for three months in the laboratory. The data are given in Table V. It will be observed that the original water resistance values for the samples containing copper hydroxynaphthenate were higher than those for the untreated control, whereas the values for the other three compounds were lower, this being particularly noticeable in the samples containing more than 0 1% copper. In the case of copper oleate, there was a marked decrease in water resistance with increase in copper content. The water resistance of the samples containing naphthenate, oleate, and tallate increased as the result of weathering and, to a lesser degree, on storage, whereas that of the hydroxynaphthenate decreased. The water resistance of all samples after exposure, with exception of those containing 0 36 and 0 76% copper as copper oleate, were at least equal to that of the original untreated sample.

(5) Cuprammonium Fluidity

It will be noted from Table VI that the fluidity tends to increase with increase in copper concentration, except in the sample containing copper hydroxynaphthenate. While the rise in fluidity shown by the other three compounds roughly parallels the breaking strength loss, the increase in fluidity shown by the samples does not appear to be of the same magnitude as the breaking strength loss. This loss in strength cannot be attributed to micro-biological attack during exposure. It had been thought that the loss may have

TABLE V

EFFECT OF WEATHERING AND STORAGE ON THE WATER RESISTANCE OF WAXED FABRICS

Sample	Treating compound	Copper, %	Waterproofness, cm. pressure to cause leakage			Change, cm.	
			Original	After storage 3 months	After exposure 3 months	After storage	After exposure 3 months
PUN	Nil	0.0	71	79	77	+ 8	+ 6
PN1	Copper naphthenate	0.093	67	70	85	+ 3	+18
PN2		0.21	54	62	71	+ 8	+17
PN3		0.37	62	70	73	+ 8	+11
PN4		0.62	53	69	85	+16	+32
PHN1	Copper hydroxy- naphthenate	0.077	92	93	75	+ 1	-17
PHN2		0.17	80	78	73	- 2	- 7
PHN3		0.32	91	80	71	- 9	-20
PHN4		0.74	92	76	76	-16	-16
POL1	Copper oleate	0.074	60	69	78	+ 9	+18
POL2		0.25	44	54	77	+10	+33
POL3		0.36	39	47	67	+ 8	+28
POL4		0.76	31	35	50	+ 4	+19
PT1	Copper tallate	0.088	80	82	77	+ 2	- 3
PT2		0.22	66	67	77	+ 1	+11
PT3		0.41	65	70	76	+ 5	+11
PT4		0.83	61	61	75	0	+14

been the result of flexing of the samples by the wind during exposure. However, more recent data have shown that similar losses are obtained with samples held taut during exposure. This effect is receiving further study.

Discussion of Data

It is of interest to refer to a few points arising out of the above data, and in this connection attention is drawn to an apparent anomaly in the breaking strength data in Table II. It will be noted that, except with the samples containing approximately 0.1% copper in the form of naphthenate and hydroxynaphthenate, soil burial following exposure did not result in any marked increase in breaking strength loss above that produced on exposure alone, although soil burial of the unleached original samples resulted in marked loss in strength in the hydroxynaphthenate, oleate, and tallate treated samples. It would thus appear that as a result of exposure the samples had become more resistant to microbiological attack. This effect is also shown by the untreated control which lost 36.9% of its strength as a result of exposure, 73.2% on exposure followed by soil burial, and 96.5% as a result of soil burial alone. The effect is also shown by the proofed samples. It is possible that this effect is connected with the presence in the fabric of non-cellulosic materials—e.g., waxes, pectic substances, traces of salts, sugars,

TABLE VI
EFFECT OF WEATHERING ON CUPRAMMONIUM FLUIDITY OF UNWAXED SAMPLES

Sample	Treating compound	Copper, %	Fluidity		Increase in fluidity
			Original	After exposure	
Untreated					
N1	Copper naphthenate	0.096	3.1	8.6	5.5
N2		0.31	4.9	9.5	4.6
N3		0.48	4.8	11.9	7.1
N4		0.48	4.1	13.9	9.8
HN1	Copper hydroxynaphthenate	0.98	4.3	14.9	10.6
HN2		0.13	3.7	9.5	5.8
HN3		0.31	3.8	9.8	6.0
HN4		0.64	3.7	10.2	6.5
OL1	Copper oleate	1.10	3.7	10.3	6.6
OL2		0.090	3.8	11.7	7.9
OL3		0.32	4.8	14.3	9.5
OL4		0.58	4.6	14.4	9.8
T1	Copper tallate	1.13	4.6	14.7	10.1
T2		0.072	4.1	10.5	6.4
T3		0.34	4.3	11.5	7.2
T4		0.54	4.1	12.9	8.8
		1.08	6.4*	14.4	8.0

* Difficult to completely remove copper tallate by solvent, possibly owing to oxidation of unsaturated constituents.

NOTE: Cuprammonium fluidity expressed as reciprocal poises.

etc., which tend to promote the growth of cellulose-destroying organisms present in the soil. Reference to the effect of such non-cellulosic materials in promoting the growth of fungi on cotton fabrics has been made by Fargher (5), who stresses the desirability of complete scouring in the case of cotton fabrics to be used under conditions conducive to microbiological attack.

It will be seen that burial of the original samples which had been previously leached resulted in a loss of strength much greater than that of the unleached samples. A recently completed study of this effect (2) has shown that copper naphthenate treated fabrics that have been subjected to sustained contact with water—e.g., soaking in running water for 24 hr.—lose much of their resistance to attack by soil micro-organisms. This effect appears to be associated with the conversion of a portion of the copper compound into a solvent-insoluble form, probably as a result of hydrolysis. It is probable that this effect also occurs with other copper soaps.

The data for the water resistance of the proofed samples are of interest in view of the marked changes in water resistance resulting from weathering and storage.

It may be mentioned that the effect of copper naphthenate in reducing the water resistance of the proofed samples is in agreement with observations made by commercial proofers.

Attention is drawn to the beneficial effect of the proofing compound in reducing the loss in strength and loss of copper resulting from weathering, and also in increasing the resistance of the samples to microbiological attack.

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PRECISION OF ASSESSMENT OF PALATABILITY OF FOODSTUFFS BY LABORATORY PANELS¹

By J. W. HOPKINS²

Abstract

Two categories of quality assessment of foodstuffs by panels of judges may be distinguished. In 'grading', an absolute assessment representative of the generality of consumers is sought. In 'analysis', maximum sensitivity is desirable and emphasis is shifted from absolute to relative assessments. In four series of 'grading' tests, individual ratings were most variable in the quality region close to the lower limit of acceptability, thus increasing to about 30 the calculable size of panel required to distinguish differences of the order of 5% from an assigned standard. The threshold concentration of primary taste substances detectable varies considerably between individuals, but except in extreme cases no consistent relation between taste acuity alone and palatability judgments was indicated. However, the judging characteristics of individuals may be investigated numerically by computing the correlation coefficients and regression equations relating their assessments to the average of those of all other members of the same panels. In this way a range of sensitivity of the order of 40% was demonstrable in the tests under review, making possible an objective evaluation of the suitability of individuals for 'grading' or 'analytical' tests.

Introduction

War conditions necessitated standardization and control of the quality of a number of bulk-produced foodstuffs, resulting, in certain instances, in increased requirements for assessment of their palatability in numerical terms. It is generally agreed that such assessments must be based in the last analysis on the reactions of human judges, for as has been pointed out by Platt (8), although the *quantity* of specific attributes of taste, odour, or texture may be capable of objective measurement, their *desirability* can only be determined subjectively. On the question of the precision of subjective assessments, however, opinion diverges widely. Thus whilst Crocker (4) states that "a considerable degree of reproducibility may be obtained in organoleptic testing" and that "even persons of ordinary discrimination may become good flavour discriminators if adequately trained", Crist and Seaton (3) conclude that "the ordinary tasting-panel method, as tested by the criterion of correlation in trials by duplication, is questionable. Either its improvement or its abandonment appears to be necessary and imperative".

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² Biometrician.

Platt (8) draws a sharp distinction between assessments based on systems of scoring by a few specially trained or experienced individuals on the one hand, and direct tests of consumer preference on the other, the latter consisting of simple comparisons made by a large number of unselected people representative of the public providing the market for the product in question. Laboratory tests may themselves be subdivided in a similar manner, depending on the nature of the information sought. A commercial laboratory, for example, may wish to use a panel of tasters to grade the output of an already established product in order to prevent loss of goodwill through the sale of material deviating noticeably from the standard to which the public is accustomed. Such a panel need not comprise highly trained or expert personnel, but should consist of a small number of individuals who could be relied on to reproduce consistently the average reaction of the consuming public. What is desired here is in fact a reliable but rapid and inexpensive gauging of consumer tolerance, and excessive sensitivity would defeat this object. If on the other hand new or modified processes are being explored, laboratory palatability tests may be regarded as essentially analytical in nature. Greater sensitivity may therefore be desirable, and emphasis may be shifted from the absolute to the relative assessment of quality. Any such enhanced sensitivity must however still be in the right direction, i.e. a substance rated above-average by a panel of this type should also appeal to the general public. These considerations may be expressed in statistical terminology by stating that the taste reactions of individuals selected for laboratory panels for both grading and analytical work should be highly correlated with those of the generality of consumers. Furthermore, the average coefficient of regression of the scores assigned by individuals on the mean values resulting if all consumers made a similar test should be in the neighbourhood of unity for a grading panel. On the other hand, a high regression coefficient should be a condition of membership in an analytical panel. Clearly, the ratings of an analytical panel may always be used for grading purposes by the application of an appropriate conversion factor, but, in an institution undertaking any volume of organoleptic testing, it might be found desirable to reserve the more sensitive judges for discriminations of which the majority of the staff were incapable.

In practice, of course, perfect correlation is unattainable, but if the magnitude of the discrepancies between individuals is known, the size of panel required to produce an average assessment of specified accuracy may be calculated. A certain amount of information bearing upon this and the preceding points has accumulated from palatability tests made in these laboratories. This has accordingly been summarized and is reported below.

Analysis of Observations

Detection of Primary Tastes

Having regard to the demonstration by Blakeslee (1) and others of hereditary deficiencies in tasting ability, Knowles and Johnson (7) subjected 37 ran-

domly chosen prospective members of a palatability panel to a trial of their sensitivity to the four primary tastes, and reported the molar dilution in which each individual was able to identify the test substances used. Two men and two women failed to detect the sweet taste in the highest test concentration of sucrose, one woman could not distinguish salt from sweet, and another was unable to identify bitter. A parallel trial was subsequently undertaken in these laboratories in which similar test solutions were submitted to 56 individuals. However, whereas Knowles and Johnson's subjects comprised 19 men and 18 women, in the later Ottawa test males predominated in the ratio of 42 men to 14 women. Two of these 42 men could not detect bitter in the highest test concentration (0.05 *M* caffeine), three failed to detect the sourness of 0.003 *M* glutamic or tartaric acids, and one was unaffected by 0.05 *M* sucrose. There was no case of complete 'taste blindness' among the 14 Ottawa women, but both sexes provided instances of incorrect identification of taste stimuli. This was most pronounced in the case of tartaric acid (16 individuals), a circumstance in agreement with the observation of Knowles and Johnson (7) that "Bitter-sour indiscriminations in the low concentrations were frequent".

Both the Knowles and Johnson and Ottawa data were subjected to statistical analysis employing the probit-log dosage transformation found applicable to a wide range of physiological and psychological responses to a graded series of stimuli. As was pointed out by Bliss (2), when the results for successive concentrations are merely different observations on a single set of individuals, the percentage reacting to a specified concentration can never be less than that recorded for a lower concentration, and successive observations are strongly correlated with each other; methods of computation appropriate to this situation were originally evolved by him for the analysis of time-mortality data in toxicological studies. By this analysis it was found that in both groups of tasters the logarithms of individual threshold concentrations were approximately normally distributed. Furthermore the median thresholds, at or below which 50% of individuals reacted, computed for the two groups separately and listed in Table I, agreed closely except in the case of caffeine (bitter), for which the observed difference exceeded three times its standard error. This may reflect a real divergence in the sensitivity

TABLE I
COMPUTED MEDIAN THRESHOLD CONCENTRATIONS (MOLAR)

Trial	Sucrose (sweet)	Caffeine (bitter)	Glutamic acid (sour)	Tartaric acid (sour)	Sodium chloride (salty)
Knowles and Johnson (North Dakota)	0.0192	0.0008	0.0010	0.00026	0.0199
National Research Labora- tories (Ottawa)	0.0195	0.0018	0.0008	0.00020	0.0192

of the tasters, but in view of the otherwise close agreement, the possibility of some lack of uniformity in the caffeine solutions used in the two tests cannot be excluded.

The variance of individual threshold concentrations in both groups and for all the test substances was of the same order, resulting in an average standard deviation of 0.3170 logarithmic units. In absolute units therefore, one would in general expect about 25% of all individuals to have thresholds exceeding the average in the ratio of 1.6 : 1 or more, whilst the median threshold of all persons below average in sensitivity would exceed the median of those of above-average sensitivity in the ratio of 3.3 : 1. Classification of the data from both laboratories in 2×2 contingency tables and calculation of the indices of association χ_c (6) listed in Table II revealed a significant degree of correlation in the sensitivity of the same individual to the sour, salt, and sweet

TABLE II

ASSOCIATION BETWEEN SENSITIVITIES TO DIFFERENT PRIMARY TASTES AS INDICATED BY χ_c FOR 2×2 CONTINGENCY TABLES

Test substance	Glutamic acid (sour)	Sodium chloride (salty)	Sucrose (sweet)
Caffeine (bitter)	2.16*	0.01	0.17
Glutamic acid (sour)		3.31**	3.23**
Sodium chloride (salty)			2.20*

* χ_c attains 5% level of statistical significance.

** χ_c attains 1% level of statistical significance.

test substances. Sensitivity to bitter was associated feebly with that to sour, and not at all with that to salt and sweet. In the same way, the one statistically significant value in Table III provided some suggestion that females detected glutamic acid more readily than did males, but provides no evidence of any effect of the smoking habit on taste acuity.

TABLE III

VALUES OF χ_c FOR 2×2 CONTINGENCY TABLE CLASSIFICATIONS OF INDIVIDUALS BY SEX AND ADDICTION TO TOBACCO, AND SENSITIVITY TO PRIMARY TASTES

Test substance	Males and females	Smokers and non-smokers
Caffeine (bitter)	0.03	1.27
Sucrose (sweet)	1.37	0.01
Glutamic acid (sour)	2.48*	0.01
Tartaric acid (sour)	1.39	0.00
Sodium chloride (salty)	1.69	0.01

* χ_c attains 5% level of statistical significance.

Knowles and Johnson (7) concluded that their results showed the necessity of testing the tasting ability of individuals before making selections for a panel of judges, and suggested criteria for the discrimination of "excellent", "good", and "fair" sensitivity, the last requiring the distinguishing of three of the four primary tastes in average or lower concentrations and eventual identification of the remaining taste. It must be agreed that it is difficult to imagine an individual notably deficient in sensitivity to two or more of the primary tastes functioning effectively as a judge of palatability. However, it must also be remembered that the sensation of palatability results from a combination of gustatory, olfactory, and tactile perceptions, the outcome of which is further conditioned by the subjective reaction of each individual to these various stimuli. Except in extreme cases, therefore, it would be too much to hope that this could be predicted from a single basic test.

Primary Tastes in Flavour Tests.

In order to provide some information respecting the extent to which sensitivity to the primary tastes affected judgments of flavour, a special series of samples consisting of scrambled egg containing various amounts of the foregoing test substances were made up and submitted to 30 of the 56 individuals taking part in the sensitivity determinations in the Ottawa laboratory. These special samples were tasted in sets of four, comprising an unadulterated fresh egg control and three preparations of fresh egg containing one of the test substances in an amount (calculated on a moisture basis) below, approximately equal to or definitely above the median threshold in the preceding trial. Such sets of four samples were included without identification in an extended series of flavour tests of scrambled egg made from dried egg powders, and were accordingly allotted scores by the tasters on the integral scale of 0 to 10, then used to assess the latter. In this way each of the 30 individuals participating tasted 20 special samples, of which five were controls and 15 were mixtures as described.

When analysed according to the procedure of Fisher (5), the total variance of the resulting 600 scores yielded the components shown in Table IV. These demonstrate that the average scores assigned by individual tasters to the entire series of samples varied appreciably, but that at the same time some statistically significant discrimination between the groups of samples containing different test substances, and between concentrations of these substances, was effected. The last component of variance shown in Table IV may be regarded as a measure of the basic error of tasting, namely inconsistency in the scoring of successive samples of the same material, and may be supposed to depend on acuity of perception and constancy of subjective reaction only. Relative scores allotted to samples actually differing in quality, on the other hand, involve judgment as well as perception, and consequently might be expected to exhibit more individual fluctuation. That this was not forthcoming in the present series of observations (Table IV) may have been because the differences in flavour between these test samples, although statistically demonstrable by reason of the large number of tasters

TABLE IV

ANALYSIS OF VARIANCE OF TASTERS' SCORES—FRESH EGG WITH AND WITHOUT ADDED SUBSTANCES

Source of variance	Degrees of freedom	Mean square
Between groups of samples	5	8.98**
Between samples of fresh egg	4	1.12
Between concentrations of added substances (average for all substances and tasters)	2	19.97**
Differential reaction to concentrations of individual substances (average for all tasters)	8	3.04
Between tasters (average for all samples)	29	26.56**
Differential reaction of individual tasters to groups of samples	145	1.69
Differential reaction of individual tasters to concentration of added substances	290	1.87
Differential reaction of individual tasters to samples of fresh egg	116	1.88

** Exceeds mean square residual, 1% level of significance.

employed, were not pronounced, and in fact corresponded to an average of less than 1 unit on the scale of assessment adopted (see Tables V and VI).

It is to be observed from Tables V and VI that, excepting one anomalous result with sucrose, the high concentration of the added substances alone was detected with any consistency, and resulted in an average reduction of palatability rating of 0.7 units. Divergence of the scores allotted to the same materials by different tasters (Table V) was however of an altogether greater order of magnitude, individuals' averages for the five control samples of fresh egg ranging from 10.0 to 5.4, and for all 20 test samples from 10.0 to 5.6. The individual (No. 10 in Table V, a male aged 39) who bestowed the maximum score on all samples indiscriminately was, as might be expected, relatively insensitive to all the primary tastes, his recorded thresholds for the test solutions exceeding the medians listed in Table I in the ratio of 4.4 for bitter, 1.3 for sweet, 2.5 for sour, and 1.6 for salty. However, taster No. 8 (a 26-year-old female) who reacted most unfavourably to the test samples, although of more than average sensitivity to sour and sweet, had an above-median threshold for bitter and was unable to identify the salty solution in the preceding trial; and, in general, correlation coefficients ranging from +0.02 (sweet) to +0.28 (bitter) were indicative of no significant association between an individual's logarithmic threshold as given by the primary test solutions and the scores subsequently allotted by him to the groups of egg samples containing the same taste substances. This finding is in agreement with the consideration, already noted, that gustatory perception is only one of several factors influencing palatability judgments.

Discrepancies between individuals' scoring of the same samples affects the reliability of palatability tests of the grading type, for in these an absolute score is required, and the panel of judges must accordingly be regarded as a theoretically random sample of the general consuming public. In self-

TABLE V
AVERAGE SCORES ALLOTTED TO GROUPS OF SAMPLES BY INDIVIDUAL TASTERS

Taster No.	Fresh egg (5 samples)	Fresh egg with addition of:					Average (20 samples)
		Sucrose (3 samples)	Caffeine (3 samples)	Glutamic acid (3 samples)	Tartaric acid (3 samples)	Sodium chloride (3 samples)	
1	7 6	8 0	7 3	8 3	7 7	7 7	7 7
2	8 0	2 3	8 0	6 7	8 0	8 3	7 0
3	8 2	8 3	8 0	7 7	7 3	8 3	8 0
4	8 8	8 7	5 3	8 0	6 7	8 3	7 5
5	9 4	8 3	10 0	9 3	9 3	10 0	9 4
6	8 8	8 3	7 7	7 7	8 0	8 7	8 3
7	10 0	8 7	10 0	10 0	10 0	9 3	9 7
8	5 6	4 7	5 7	6 7	5 0	5 7	5 6
9	8 0	7 7	8 7	7 7	7 7	7 7	7 9
10	10 0	10 0	10 0	10 0	10 0	10 0	10 0
11	7 0	6 7	7 3	6 0	7 7	7 0	7 0
12	8 2	7 7	8 3	8 7	9 0	8 3	8 4
13	7 2	4 3	4 0	6 3	7 3	7 7	6 3
14	7 6	6 0	6 7	8 0	8 0	6 0	7 1
15	10 0	8 0	10 0	9 3	8 3	10 0	9 4
16	8 0	6 0	5 0	7 3	7 7	8 3	7 2
17	7 8	8 0	7 3	7 7	7 3	7 3	7 6
18	5 4	6 3	5 7	7 3	5 3	8 3	6 1
19	8 4	6 3	8 7	6 0	7 3	8 0	7 6
20	9 4	10 0	10 0	9 7	8 7	9 7	9 6
21	6 0	7 0	5 7	6 3	5 0	6 0	6 0
22	10 0	8 7	8 7	9 3	9 7	9 3	9 4
23	8 8	7 7	8 3	8 3	8 3	9 0	8 5
24	6 8	5 0	5 3	8 3	6 3	8 7	6 8
25	9 4	8 0	7 3	7 7	8 6	7 3	8 2
26	8 8	8 3	8 3	8 7	7 3	9 3	8 5
27	8 0	7 7	8 0	7 7	8 0	7 0	7 7
28	7 0	8 0	6 7	7 3	8 0	7 3	7 4
29	6 2	7 7	6 7	7 3	6 7	7 7	7 0
30	7 4	8 3	6 3	7 7	9 0	8 0	7 7
Average	8 1	7 4	7 5	7 9	7 8	8 1	7 8

NOTE. Necessary difference between averages of fresh egg and other groups = 0.36.

Necessary difference between averages of other groups = 0.40.

Necessary difference between averages of individual tasters = 0.9.

contained analytical tests on the other hand, if an entire series of samples is assessed by the same judges, average differences between the ratings given by individuals to the series as a whole do not enter into comparisons of the relative scores given to specific samples. These are affected only by errors arising from sources of the type itemized in the last three lines of Table IV.

Variance of Palatability Assessments in Relation to Quality Level

It is of evident practical importance to know whether the variance of individual judgments of palatability, as expressed in numerical scores, is uniform over the range of quality encountered, and also whether some materials are productive of greater disagreement between individuals than others. Table VII summarizes the experience of this laboratory relevant to these points

TABLE VI
EFFECT OF CONCENTRATION OF ADDED SUBSTANCES ON SCORE (AVERAGE
OF 30 TASTERS)

Substance	Concentration			
	Zero	Low	Medium	High
Sucrose	8.1	6.9	7.8	7.4
Caffeine	8.1	7.7	7.8	7.0
Glutamic acid	8.1	7.9	8.2	7.6
Sodium chloride	8.1	8.4	8.5	7.3
Tartaric acid	8.1	7.8	8.1	7.5
Average	8.1	7.7	8.1	7.4

NOTE: Necessary difference between averages = 0.3.
 Necessary difference between individual items and zero = 0.5.
 Necessary difference between individual items other than zero = 0.7.

TABLE VII
STANDARD DEVIATION OF INDIVIDUAL ASSESSMENTS AT VARIOUS QUALITY LEVELS

Test substance	Statistic	Average score									
		10 0- 9.1	9 0- 8.1	8 0- 7.1	7 0- 6.1	6 0- 5.1	5 0- 4.1	4 0- 3.1	3 0- 2.1	2.0- 1.1	1.0- 0.0
Butter (Panel of 17)	Standard deviation	—	0.84	1.15	1.23	1.30	1.49	1.62	1.63	1.11	0.84
	No. of samples	—	1	45	65	52	57	30	15	11	3
Dried eggs (Panel of 6)	Standard deviation	0.59	0.83	1.14	1.38	1.66	1.66	1.54	2.17	1.97	—
	No. of samples	2	41	108	139	64	59	19	10	2	—
Dried milk (Panel of 14)	Standard deviation	—	—	0.97	1.12	1.22	1.35	1.50	1.49	—	0.59
	No. of samples	—	—	3	60	90	70	27	12	—	2
Ration biscuits (Panel of 16)	Standard deviation	—	1.10	1.25	1.36	1.61	1.76	1.84	1.56	1.98	—
	No. of samples	—	28	190	206	160	73	17	2	1	—

resulting from a fairly extensive series of palatability tests of four foodstuffs, namely ration biscuits, dried eggs, butter, and dried milk. The standard deviations shown in this table are appropriate to absolute scores, i.e., to the grading type of test, and thus include any average differences between tasters.

On the whole, the degree of variability indicated was of the same order for all four substances. Furthermore, in all four cases individual scores became clearly less consistent as quality decreased, and were possibly most discrepant in the region corresponding to scores of 2 to 5. Owing to the infrequency of samples of the lowest quality, the zone of maximum uncertainty is not well determined. The existence of such a zone in any extended series including extremely good and extremely bad samples is however implicit in the bounding of the assignable scores.

It should be noted that an increase in individual variability necessitates the employment of more judges to produce an average assessment of specified reliability. In order to equalize the variance of the scores for dried egg given in Table VII for example, nearly eight times as many judges would be required in the quality range 2 to 3 as in 8 to 9. Deductions may also be made from Table VII respecting the actual number of judges required to yield average scores of specified accuracy. Thus in order to detect, at the 5% level of statistical significance (5), samples of dried egg differing from a pre-assigned quality rating of 4.0 by 1 unit or more on the quality scale here employed, 12 judges would be needed; whilst the detection of deviations of 0.5 unit would require the averaging of 46 individual scores. Discrimination between two samples of this mediocre quality rated by different panels would call for 23 judges per sample for a necessary difference of 1 unit and for 92 per sample for a necessary difference of 0.5 unit.

Correlation of Individuals' Assessments with Panel Averages

In an introductory paragraph it was pointed out that the suitability of individuals for laboratory taste panels might be evaluated statistically in terms of regression and correlation coefficients. Ideally, such evaluations should be made by relating each individual in question to the average of the consuming public. In practice, a reasonably representative sample of the latter must suffice. Even this was not available in the present instance, but, as the panels employed in this laboratory on the tests summarized in Table VII comprised scientific workers, technicians, and members of the administrative staff unselected in respect of tasting experience, training, or ability, correlation and regression coefficients of several individuals relative to all other panel members assessing the same samples have been computed as a matter of interest, and are shown in Table VIII.

Generally speaking the highest correlation coefficients resulted from the tests of ration biscuits, and the lowest from those of dried milk. However, in fairness to the tasters employed on the latter it should be noted that the great majority of samples of these fell within a narrow range of quality (see Table VII), so that discrepancies between individuals would be expected to constitute an increased proportion of the total variance. An appreciable range is to be observed in individuals' correlation coefficients for butter (0.66 to 0.88) and dried milk (0.44 to 0.68), whilst ration biscuits gave slightly more uniform results (0.74 to 0.84). A somewhat higher degree of correlation is obviously desirable, and would operate to reduce the rather large numbers of tasters specified in the preceding section for results of statistical significance. Nevertheless this aspect of the tests was by no means wholly unsatisfactory, and certainly lends no support to the pessimistic views of Crist and Seaton (3) respecting the non-reproducibility of test panel results.

The regression coefficients listed in Table VIII, which as noted above provide a measure of the sensitivity of individuals' reactions, vary from 0.72 to 1.11 for butter, from 0.69 to 1.10 for dried milk, and from 0.68 to 1.14 for ration biscuits. For all three substances therefore, the range was of the order

TABLE VIII

CORRELATION OF INDIVIDUALS' ASSESSMENTS WITH AVERAGES OF REMAINDER OF PANEL

Test substance	Taster	No. of samples	Correlation coefficient	Regression coefficient	Average deviation of assessments from all others
Butter (Panel of 17)	<i>A</i>	130	.78	0.93	-0.26
	<i>B</i>	142	.88	1.09	-0.50
	<i>C</i>	140	.87	1.16	+0.06
	<i>D</i>	136	.76	0.89	-0.04
	<i>E</i>	127	.66	0.72	-0.54
	<i>F</i>	116	.81	1.11	+0.56
	<i>G</i>	128	.77	0.80	-0.15
	<i>H</i>	147	.76	0.94	-0.87
	<i>I</i>	117	.77	1.02	+0.06
Dried milk (Panel of 14)	<i>A</i>	307	.64	1.05	+0.52
	<i>B</i>	248	.54	0.95	-1.12
	<i>J</i>	250	.67	0.91	-0.42
	<i>K</i>	263	.68	1.10	-0.17
	<i>L</i>	296	.44	0.80	-0.45
	<i>M</i>	226	.54	0.86	+0.24
	<i>N</i>	256	.66	0.90	+0.46
	<i>O</i>	189	.47	0.69	+0.97
	<i>P</i>	222	.67	1.03	+0.46
Ration biscuits (Panel of 16)	<i>A</i>	157	.83	0.91	+0.70
	<i>B</i>	157	.78	1.04	-0.55
	<i>C</i>	139	.83	1.14	+0.09
	<i>G</i>	167	.75	0.68	+0.62
	<i>J</i>	166	.84	0.94	-0.12
	<i>K</i>	167	.80	0.85	-0.12
	<i>Q</i>	147	.74	0.96	+1.19

of 40% of the mean. The last column of Table VIII shows the amount by which the average score given by each judge exceeded or fell below the average of all other panel members assessing the same samples, and provides some further examples of consistent differences in individual preference levels of the type illustrated in Table V. The correlation coefficient, regression coefficient, and average score together provide a numerical characterization of the performance of the various judges. Taster *C* in Table VIII, for example, with a correlation coefficient of 0.87, a regression coefficient of 1.16, and an average preference level differing by only +0.06 from the remainder of the panel would be an obvious choice for the analytical assessment of butter, whilst *E*, with a correlation coefficient of 0.66, a regression coefficient of 0.72, and a mean deviation from the average preference of -0.54 scale units, equally obviously would not. Taster *C* also had the highest regression coefficient of those members of the ration biscuit panel investigated. Taster *B* had regression coefficients in excess of unity for both butter and ration biscuits but also exhibited a mean deviation from the average preference level of about -0.5 units in both instances, again illustrating the necessity of maintaining panel personnel unchanged throughout any series of samples for which maximum precision of relative assessment is desired.

Conclusions

The results described above may be summarized as follows:

There is appreciable variation in the ability to detect primary taste substances, the logarithms of individual threshold concentrations being approximately normally distributed. However, assessments of the palatability of foodstuffs depend upon olfactory and tactile as well as upon gustatory sensations, and are further conditioned by the subjective reaction of individuals to these stimuli. Except in extreme cases therefore, no consistent relation between taste acuity alone and palatability judgments is to be anticipated.

Two categories of quality assessment of foodstuffs by panels of judges may be distinguished. In the 'grading' type of test an absolute assessment is sought, and the panel must provide a representative sample of the reaction of the generality of consumers to the product in question. In tests of the 'analytical' type the effects of new or modified processes are compared, maximum sensitivity is desirable, and emphasis is shifted from absolute to relative assessments.

Analogous considerations apply to the fluctuations affecting the reproducibility of assessments, and a distinction may be made between consistent differences in preference level on the one hand, and the differential reaction of individual judges to particular samples on the other. Providing an entire series of samples is assessed by the same judges, the former may be eliminated from comparisons made within a test of the 'analytical' type. They cannot however be excluded from assessments on an absolute scale, in which they must be expected to constitute a major source of error.

In a series of tests in this laboratory in which quality was assessed on a numerical scale of 0 to 10, individual ratings became progressively more erratic as quality deteriorated, and were judged to reach a maximum of variability in the quality region corresponding to average scores of 2 to 5. This circumstance has the effect of increasing considerably the size of panel required to produce results of statistical significance when grading products of a quality close to the lower limit of acceptability.

The assessment characteristics of individuals may be investigated numerically by calculation of the coefficients of correlation and regression relating their scores to the average of those of all other members of the panel, and also by determining the mean amount by which their preferences are in excess or defect of the remaining panel average. In the tests considered here there was appreciable variation in both the correlation and regression coefficients of individuals, the latter indicating a range of sensitivity of the order of 40% of the mean. By statistics such as these the suitability of individuals for grading or analytical panels may be objectively evaluated, although in the case of grading panels it must always be borne in mind that the aim is to produce assessments characteristic of the consuming public in general rather than of the laboratory staff in particular.

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DRIED WHOLE EGG POWDER

XX. THE EFFECT OF GRADE OF EGGS, LOCALITY AND MONTH OF PRODUCTION, AND CLIMATIC CONDITIONS ON THE SOLIDS CONTENT OF LIQUID EGG AND ON THE QUALITY OF THE POWDER PRODUCED¹

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Abstract

Grade *A* large eggs had the lowest average solids content (25.6%) of the grades studied and Grade *C* eggs had the highest (26.6%). The solids content increased during the period from December 1944 to July 1945. The total increase during this interval was about 0.5%.

The average potassium chloride value of powder produced from Grades *B* and *C* eggs was higher than the value for Grade *A* medium eggs; Grade *A* medium and pullet eggs produced powder better in this quality attribute than Grade *A* large (differences of about 2%). The use of Grade *C* eggs resulted in a powder with an average fluorescence value about 2 units greater than for powder from any other grade of eggs. The month of egg production affected quality measures on the resulting powder. As the season progressed there was a decrease in the fluorescence value and pH and an increase in potassium chloride value and foaming volume value.

The changes noted could not be attributed to climatic conditions, but may be attributable to feeding practices and to increased age of the hen.

Introduction

The increased production of dried eggs in Canada has focused attention on possible differences between lots of eggs that may affect both the yield and quality of the resulting powder. Recurring statements from some producers of powder indicated that eggs from different parts of the country varied widely in solids content. One processor claimed that eggs from one area consistently produced 4% less powder than eggs from another area. Therefore, it seemed advisable to attempt a monthly assessment of the solids content of various grades of eggs from different parts of the country.

There is some evidence that eggs produced between November and March are of the highest quality (3, 4, 5, 7, 12). Although these reports did not deal with solids content specifically, they describe decreases in quality from March to November as assessed by the condition of the thick white, the percentage of thick white, yolk index (4, 5), and candling appearance (7). There was no seasonal change in yolk colour (4, 5) but this may be attributed to the use of standard mash for feeding the experimental birds. One Canadian report indicates that the albumin index of eggs produced in March is higher than that of eggs produced in June (3).

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Therefore, simultaneously with an assessment of solids content of the liquid eggs, some of the material was dried and the yield determined. The properties of the powder were assessed, using the quality tests currently applied to dried egg powder (6) and a test of baking properties (9). The colour of the powder was measured on a colour comparator (13). The present paper describes differences in solids content of the liquid eggs, and in yield and quality of the dried product. The colour measurements will be discussed as part of a subsequent paper on the colour of dried egg powder (10).

Materials and Methods

The original experimental design required the shipment of eggs from Vancouver, Edmonton, Winnipeg, London, Belleville, and Three Rivers. While these did not represent all the areas from which eggs are purchased for drying, they did represent the areas subject to controversy and were believed to be a good cross-section of Canada's egg producing districts. Since surplus eggs become available in Canada in December and production falls off the following July when the hens begin to moult, eggs were taken from current receipts on the 15th of each month from December 1944 to July 1945. This would represent the eggs most likely to be dried. Five dozen of each of the following grades were to be shipped at each sampling time: *A* large, *A* medium, *A* pullet, *B* and *C*. Since Canadian eggs can be classed as *B* and *C* for dirty shells as well as for quality (2), the samples representing these grades were selected on the basis of quality only. All eggs were to be drawn from current receipts in the areas designated, and samples were taken and graded by inspectors of the Special Products Board of the Canadian Department of Agriculture.

Unfortunately, the original plan could not be adhered to entirely. Only four areas could supply eggs in December 1944, and it was impossible to obtain the required *B* and *C* eggs from the Vancouver area. From time to time, single items were missing in the various consignments. Nevertheless, it was believed that the survey was sufficiently complete to permit conclusions of value. Altogether, 224 five-dozen lots of eggs were used in the study.

Upon receipt in the laboratory, the eggs of each of the various grades were broken and mixed; samples were then drawn for determinations of solids content (1, p. 308) and the remainder was weighed before going to the laboratory spray drier (14) for dehydration (drying temperatures: inlet, 250° F.; outlet, 140° F.). After dehydration the material from the main chamber and the secondary collector were weighed separately, and the moisture contents determined (11). From these values, the recovered solids were calculated as a percentage of the solids introduced into the drier.

Only the powder from the main chamber was used for measurement of fluorescence value (8), potassium chloride value (11), pH (6), and foaming volume (9).

Results

The significance of the results was assessed by analyses of variance. Two comparisons were made: the first compared Grade *A* eggs of all sizes from the six areas for the months of January to July 1945; the second compared Grade *A* medium eggs with Grades *B* and *C* eggs for the same collection times but for five areas only. Since the mathematical treatment showed little evidence of differential behaviour, the presentation of the results has been simplified by the use of tables of means and necessary differences (Tables I, II, and III). The effect of area of production on solids content of Grade *A* eggs almost attained significance and was given further consideration (Table IV). There appeared to be some relation between quality and time of production and possibly some relation between quality and climatic conditions, shown by tables of correlation coefficients (Tables V and VI).

Solids Content and Yield

The results indicated that Grade *A* large eggs had a significantly lower solids content than Grade *A* medium and Grade *A* pullet eggs, while Grade *C* eggs had the greatest solids content (Table I). Area of production had a significant effect on the solids content of Grades *B* and *C* eggs and the effect almost attained significance for Grade *A* eggs (Table II). Although the differences are small, there is generally a tendency for solids content to increase as the season progresses (Table III). This would correspond to a previously observed seasonal trend in loss of quality before the candling lamp (7). One particular feature is increase in the size of the air cell that is believed to accompany loss of moisture through the shell.

TABLE I

RELATION BETWEEN GRADE OF EGGS, SOLIDS CONTENT, AND QUALITY OF EGG POWDER PRODUCED DURING A SEVEN MONTH PERIOD

Grade of eggs	Solids content, %	Quality of powder			
		Fluorescence value	Potassium chloride value, %	pH	Foaming volume, ml.
Averages for eggs produced in six areas					
A large	25.6	23.1	65.8	8.76	235
A medium	25.9	21.3	66.6	8.75	247
A pullet	25.9	21.8	68.0	8.67	241
Necessary difference, 5% level	0.3	1.6 ¹	1.8	0.09 ¹	24 ¹
Averages for eggs produced in five areas					
A medium	26.0	21.3	67.0	8.73	247
B	26.2	22.4	69.5	8.67	247
C	26.6	24.6	69.7	8.59	243
Necessary difference, 5% level	0.3	1.4	1.7	0.13 ¹	20 ¹

¹ Difference not significant.

TABLE II

RELATION BETWEEN AREA IN WHICH EGGS ARE PRODUCED, SOLIDS CONTENT, AND QUALITY OF EGG POWDER PRODUCED DURING A SEVEN MONTH PERIOD

Area of production	Solids content, %	Quality of powder			
		Fluorescence value	Potassium chloride value, %	pH	Foaming volume, ml.
<i>Averages for Grade A eggs</i>					
Vancouver	25.7	23.3	64.9	8.75	242
Edmonton	25.6	21.4	68.5	8.74	252
Winnipeg	25.5	22.4	66.2	8.78	242
London	25.7	22.5	66.7	8.73	239
Belleville	25.8	20.4	68.2	8.74	250
Three Rivers	26.0	21.9	65.4	8.67	236
Necessary difference, 5% level	0.4 ¹	2.2 ¹	2.6	0.13 ¹	33 ¹
<i>Averages for Grades A medium, B, and C eggs</i>					
Edmonton	26.7	23.4	70.8	8.60	249
Winnipeg	26.0	22.8	67.5	8.78	253
London	26.1	21.9	69.3	8.70	238
Belleville	25.8	21.3	67.5	8.66	251
Three Rivers	26.5	23.8	67.8	8.59	238
Necessary difference, 5% level	0.4	1.9 ¹	2.2	0.17 ¹	25 ¹

¹ Difference not significant.

The effect of area of production on the solids content was given further consideration (Table IV). This table indicates a greater proportion of eggs of low solids content from Western Canada. However, Grade C eggs from Edmonton had high solids contents (average, 27.8%), thus accounting for the high value noted in the second part of Table II.

The only differential behaviour of significance was noted for eggs from Belleville. Grade C eggs from this area had, on the average, lower solids content than Grade B eggs, which in turn had a lower solids than Grade A mediums. The average values were: Grade C, 25.5%; Grade B, 25.8%; Grade A medium, 26.0%. No explanation can be offered to show why these results do not conform to other data presented in this paper or to everyday experience.

Calculations on powder yield in relation to solids entering the drier showed no differences in drying characteristics as a result of differences in grade, area, or time of production. The average recovery on the model drier was 92%. Although the distribution curve was slightly skewed, the standard deviation was calculated as 6%, which gives some indication of the variability observed.

TABLE III

RELATION BETWEEN MONTH IN WHICH EGGS ARE PRODUCED, SOLIDS CONTENT, AND QUALITY OF THE POWDER PRODUCED

Month of production, 1944-5	Solids content, %	Quality of the powder			
		Fluorescence value	Potassium chloride value, %	pH	Foaming volume, ml.
<i>Averages for Grade A eggs produced in six areas</i>					
December ¹	25.4	21.9	61.2	8.96	223
January	25.6	24.0	64.0	8.88	234
February	25.6	24.8	66.4	8.74	219
March	25.7	21.5	69.3	8.73	265
April	25.9	22.5	65.9	8.74	237
May	25.8	21.8	65.6	8.76	232
June	26.2	21.6	71.7	8.78	285
July	26.0	17.9	66.7	8.51	235
Necessary difference, 5% level	0.5	2.4	2.8	0.14	36
<i>Averages for Grades A medium, B, and C eggs produced in five areas</i>					
December ¹	26.2	22.3	61.6	8.88	223
January	26.2	24.9	64.9	8.80	234
February	26.2	26.1	66.1	8.66	220
March	26.1	20.6	69.0	8.72	251
April	26.2	22.1	69.8	8.77	248
May	26.3	22.9	69.3	8.70	244
June	26.5	22.3	72.7	8.73	282
July	26.5	19.4	68.3	8.26	239
Necessary difference, 5% level	0.4	2.2	2.6	0.20	30

¹ Eggs from only four areas available in December. Therefore these measurements not included in analysis of variance.

TABLE IV

VARIATION IN SOLIDS CONTENT OF VARIOUS GRADES OF EGGS FROM VARIOUS DISTRICTS SAMPLED DURING AN EIGHT MONTH PERIOD

Grade of eggs	Solids content, %			Area and number of samples greater or less than standard deviation											
	Mean	S.d.	Range	Greater						Less					
				V.	E.	W.	L.	B.	T.R.	V.	E.	W.	L.	B.	T.R.
<i>Production from six areas</i>															
Grade A large	25.6	0.5	24.5 – 26.3	1	1	1	1	0	3	1	3	1	1	1	0
Grade A medium and pullet	25.9	0.5	24.6 – 27.3	0	1	0	2	1	3	5	1	0	3	1	0
<i>Production from five areas</i>															
Grade B	26.2	0.4	25.0 – 27.5	—	0	1	0	1	0	—	2	0	0	0	1
Grade C	26.7	1.0	25.5 – 29.9	—	2	0	0	0	0	—	0	0	0	2	0

The data in Table IV indicated that, in spite of fairly constant behaviour during the drying operation, variation in production is possible because of differences in solids content of the eggs. In Canada, only eggs of *B* grade or better are dried, thereby making possible variation in recovered solids of between 24.5 and 27.5 lb. per 100 lb. of liquid egg introduced into the drier. Since the quantity of liquid egg dried in one day in a commercial plant greatly exceeds the size of the samples studied here, some reduction in this variation is likely. If variations of the magnitude of the standard deviations (Grades *A* and *B* eggs, Table IV) are encountered in commercial practice, 100 lb. melange may produce between 25.1 and 26.6 lb. of powder. That is, a plant drying 40,000 lb. of liquid egg per day may recover between 10,040 and 10,640 lb. of solids. These values are more extreme than, but support, the 400 lb. differences reported from some commercial plants producing about this quantity of powder.

Quality of the Powder Produced

Neither grade of eggs nor area of production had a significant effect on the pH and foaming volume values of the powder produced, but month of production appeared to affect all quality measures (Tables I, II, and III). The use of Grade *C* eggs resulted in powders with increased fluorescence values. The high fluorescence value noted for Grade *A* large eggs was attributable to the high values for this grade of eggs from the Vancouver area. Powders produced from Grade *A* pullet eggs had higher potassium chloride values than powder from Grade *A* large eggs, and Grades *B* and *C* eggs produced powder significantly better in this quality attribute than Grade *A* mediums. Powder from Grade *A* eggs from Edmonton and Belleville areas had higher potassium chloride values than powder from similar eggs produced in the Vancouver and Three Rivers areas. The comparison of *B* and *C* with *A* medium eggs further supported the high potassium chloride values for eggs from the Edmonton area.

Time of production affected all quality attributes of the powder (Table III). Fluorescence values were high in February and decreased thereafter, while potassium chloride values progressively increased until June and decreased markedly in July (time of first moult). A regular decrease in pH was observed throughout the period studied. Foaming volume values were greatest in March and June. Since there were changes in quality with month of production, which may have been the results of climatic conditions, these changes were studied further by calculation of the correlation coefficients between quality attributes and climatic condition.

Correlations Between Time, Climatic Conditions, and Quality

Correlation of the various measurements for Grade *A* eggs or powder from Grade *A* eggs with time of production is shown in Table V. Average values for solids content and potassium chloride value and foaming value of the powder increased as the season of production progressed, while average fluorescence value and pH of the powder decreased. The only relations

attaining significance were those between time of production and solids content, and time of production and pH. Considered independently of time effects, positive correlations were observed between average solids content and average fluorescence, potassium chloride and pH values of the powder. However, these relations failed to attain significance.

TABLE V
CORRELATION COEFFICIENTS BETWEEN TIME OF PRODUCTION, AVERAGE SOLIDS CONTENT OF LIQUID EGG, AND AVERAGE VALUES FOR QUALITY MEASUREMENTS ON EGG POWDER

Items correlated	Correlation coefficient
Time correlated with averages for: (6 degrees of freedom)	
Solids content of liquid eggs	.886**
Fluorescence value of powder	-.704*
Potassium chloride value of powder	.656
pH of reconstituted powder	-.799*
Foaming volume for powder	.456
Correlation of solids content with averages for quality measures independent of time effects: (5 degrees of freedom)	
Fluorescence value of powder	.417
Potassium chloride value of powder	.558
pH of reconstituted powder	.375
Foaming volume for powder	.077

* 5% point of statistical significance, $r = \pm .707$.

** 1% point of statistical significance, $r = \pm .834$.

When considering the effects of climatic conditions (dry bulb temperature, sunshine, rainfall) it was believed that the average condition occurring during the first 15 days of the month might be correlated with the various measures, if the quality was affected by the climate to which the egg is exposed just after laying. Conversely, if climate were affecting egg quality prior to laying, the average condition occurring during the last 15 days of the preceding month might be correlated with the measures. To make the comparison, correlation coefficients were calculated independently of time and area. Only one relation attained significance (5% level) and since there were 30 comparisons this might be expected to occur by chance (Table VI). It was thought possible that significant relations might be observed if the data were broken down for periods from January to March and April to June, i.e., for periods when hens are sheltered or outside. However, even with this breakdown none of the coefficients became significant.

In general, the failure of the correlations to attain significance for any factor other than time indicates that the age of the hen may be the most important single factor responsible for the changes noted in the solids content

TABLE VI

CORRELATION COEFFICIENTS RELATING CLIMATIC CONDITIONS TO THE AVERAGE SOLIDS CONTENT OF GRADE A EGGS COLLECTED ON THE 15TH OF EACH MONTH FROM JANUARY TO JUNE AND TO THE QUALITY OF THE POWDER PRODUCED (INDEPENDENT OF TIME AND AREA¹)

Average condition during the first 15 days of the months correlated with:	Correlation coefficient (15 degrees of freedom)		
	Dry bulb temperature	Sunshine	Rainfall
Liquid egg Solids content	— .313	— .160	.354
Powdered egg Fluorescence value	.068	— .411	.196
Potassium chloride value	.195	.439	.360
pH	— .143	.280	.096
Foaming volume	.259	— .483*	— .072
Average condition during the last 15 days of the preceding months correlated with:			
Liquid egg Solids content	.187	— .145	.282
Powdered egg Fluorescence value	.008	— .345	.373
Potassium chloride value	— .030	— .353	.336
pH	— .021	.147	— .040
Foaming volume	— .045	— .306	— .255

¹Only samples taken from January to June and eggs from Three Rivers, Winnipeg, Edmonton, and Vancouver were considered in this treatment.

* 5% point of statistical significance, $r = \pm .482$.

of the liquid egg and in the quality of the powder produced. The differences noted for various areas may be attributable to local variations in feeding practice.

Acknowledgments

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THE APPLICATION OF DUST-LAYING OIL TO WOOL¹

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Abstract

A process is described for the application of dust-laying oil to woollen blankets, in which use is made of a positively charged oil-in-water emulsion. During a commercial-scale trial of this process, difficulty was encountered in the form of a progressive build-up of oil and fatty acid after repeated laundering and oiling. The inclusion in the laundering formula of modified soda in a soap : soda ratio of 3 : 1 has been found to give satisfactory control of the fatty acid content, and to assist greatly in preventing the accumulation of oil.

Introduction

The treatment of hospital bed-clothes with dust-laying oil has been shown to result in a marked reduction in the number of bacteria distributed into the air during bed-making (3). Of the several methods available for the application of oil to wool, the most satisfactory is that developed by Harwood, Powney, and Edwards (2) in which use is made of a dilute oil-in-water emulsion stabilized by a cation-active emulsifying agent. The chemical aspects of this process have been investigated by Bayley and Weatherburn (1) and a somewhat modified procedure has been proposed.

The present paper deals first with a practical trial of this process on a commercial scale. During the course of this trial difficulty was encountered in the form of an objectionable accumulation of oil and fatty acid on the wool after repeated laundering and oiling. The remainder of the paper deals with a method for overcoming this difficulty.

Experimental

The procedure, as outlined in the previous paper (1), comprises a preliminary laundering, consisting of two suds with soap, two rinses with water, and souring at pH 5.0 with sodium silicofluoride. This is followed by the oiling operation in which a stock oil emulsion is added in sufficient quantity to give the desired degree of oiling on the wool.

Preparation of Stock Emulsion

For the sake of convenience in shipping and storage, stock emulsions containing as high a percentage of oil as possible are desirable. Emulsions containing 70% of oil and 1.75% of a commercial preparation of cetyldimethylbenzylammonium chloride have been prepared and found satisfactory. Emulsions containing higher proportions of the emulsifying agent are equally stable, but an excess should be avoided, both from the standpoint of economy

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and because of the retarding effect on the rate of exhaustion of the treating bath (1).

The stock emulsion was prepared as follows:

Oil*	70% by weight
Emulsifying agent†	1.75% by weight
Water (140° to 160° F.)	28.25% by weight

The emulsifying agent was dissolved in the hot water, the oil was stirred in, and the mixture was passed through a colloid mill. This emulsion tended to "cream" on standing, i.e., to separate into two layers, the upper one of which contained a high proportion of the oil, whereas the lower one was largely water. There was no separation of clear oil, however, and, on gentle mixing, an emulsion of uniform appearance was again obtained.

The emulsion did not freeze on storage for 96 hr. at 30° F., and on being restored to room temperature, appeared to be unchanged. After storage for a similar length of time at 0° F., however, the emulsion solidified, and on subsequent melting it broke down with the separation of clear oil. Metal containers in which the emulsion was stored were found to be rusted after a short time. It is therefore necessary to use non-rusting containers, and to avoid freezing of the emulsion.

Method of Oiling

A full-scale trial was carried out in commercial laundry equipment at the Sanitary Laundry, Ottawa. This trial involved the laundering and oiling of 200 woollen blankets, divided into three batches, *A*, *B*, and *C*, containing 67, 67, and 66 blankets, respectively. Batches *A* and *B* were laundered and oiled once, while Batch *C* was given three additional treatments, making a total of four launderings and oilings for this batch.

A piece of the woollen blanket material measuring 12 by 12 in. was pinned to each of six test blankets in each load, and the loads were laundered and oiled according to the following formula:

Operation	Material	Time, min.
1. Suds	Soap	10
2. Suds	Soap	10
3. Rinse	Water	5
4. Rinse	Water	5
5. Sour	Sodium silicofluoride	5 - 10
6. Oiling	Stock emulsion	10

The water level was maintained at the rinse level, and the temperature at 100 to 110° F. during each operation. Softened water was used throughout. Dry powdered low-titre soap was added to the wheel in sufficient quantity to give a good suds.

* *Marcol HX*, supplied by *Imperial Oil Company*.

† *Triton K-60*, supplied by *Rohm and Haas Company*.

In the souring operation, dry sodium silicofluoride was added in small quantities until the pH was reduced to 5.0. For the first two loads the time of souring was five minutes. It was apparent that the souring was not complete in these loads, since the liquor in the washer at the end of operation 5 was opalescent, indicating the presence of soap, and the oil was not completely exhausted from the bath in operation 6. Accordingly, in the succeeding loads both the quantity of sour and the time of treatment were increased. Details of the souring conditions are given in Table I. It was found that approximately 1 lb. of sodium silicofluoride per 100 lb. of load and 10 min. treatment were required to ensure complete souring. The term "complete souring" may be defined as the reduction of the soap content of the blanket to an amount equivalent to, or less than, the amount of emulsifying agent added in the stock emulsion in operation 6. It has been shown (1) that under these conditions the presence of soap does not interfere with the oiling process.

TABLE I
SOURING DATA

Batch	Load No.	Weight sour per 100 lb. load, lb.	Time, min.	pH of souring liquor	Appearance of souring liquor and residual oil bath
A	1	0.30	5	5.1	Cloudy
B	2	0.37	5	4.9	Cloudy
C	3	0.90	10	5.5	Faintly cloudy
C	4	1.00	10	5.1	Clear
C	5	1.00	10	4.5	Clear
C	6	1.00	10	4.4	Clear

For the sake of convenience in measuring, the stock emulsion was diluted to a concentration of 3.85 lb. oil per gal. (132 lb. diluted to 24 gal.). Four gallons of this material was added to each load. Since the average load weight was 300 lb. the estimated oil content of the treated blankets was:

$$\frac{3.85 \times 4 \times 100}{300 + (3.85 \times 4)} = 4.9\%$$

After oiling, the blankets were hydro-extracted and dried at room temperature by hanging. Four samples for analysis were cut from the body of each test blanket, and two samples from each pinned-on test-piece.

Determination of Oil and Fatty Acid Content of Oiled Blankets

The samples were conditioned for 24 hr. at 70° F. and 65% relative humidity. A 10 to 15 gm. sample (weighed accurately) was extracted for three hours with 150 ml. of petroleum ether (boiling range 30 to 60° C.) in a Soxhlet apparatus. The ether extract was filtered into a second flask, and the first flask and filter paper were washed out with a further 25 ml. portion of petroleum ether. The excess ether was distilled from the combined extract and washings, the

residue was transferred to a weighed evaporating dish, and the flask was washed out with petroleum ether, the washings being added to the dish. The remaining ether was evaporated off on a boiling water-bath, and the heating continued for 20 min. The evaporating dish was cooled in a desiccator for 15 min. and weighed.

After weighing, the extract was dissolved in 25 ml. of 95% ethyl alcohol and titrated with *N*/10 sodium hydroxide solution, using phenolphthalein indicator. The blank on 25 ml. of the alcohol was determined and subtracted from the titration value. The weight of fatty acid, calculated as stearic, was estimated and subtracted from the total weight of extracted matter to give the weight of oil recovered.

The results of these analyses are summarized in Table II. The standard deviations were calculated in the usual manner, viz.:

$$S.D. = \sqrt{\frac{\sum X^2}{N} - \bar{X}^2},$$

where X = the result of a single determination,

\bar{X} = the mean for N determinations,

N = the number of determinations.

In Table II, the *B* samples were taken from the body of the test blankets. Each figure is an average of 24 determinations. The *T.P.* samples were taken from the pinned-on test-pieces. Each figure is an average of 12 determinations.

TABLE II
OIL AND FATTY ACID ANALYSIS OF OILED WOOL BLANKETS

Batch	Load No.	No. of oilings	Oil content, %				Fatty acid content, %			
			<i>B</i> samples		<i>T.P.</i> samples		<i>B</i> samples		<i>T.P.</i> samples	
			Av.	Standard deviation	Av.	Standard deviation	Av.	Standard deviation	Av.	Standard deviation
<i>A</i>	1	1	4.0	2.97	4.6	2.96	0.22	0.156	0.18	0.116
<i>B</i>	2	1	3.2	2.61	7.2	2.35	0.20	0.095	0.21	0.083
<i>C</i>	3	1	4.2	2.85	4.7	2.15	0.44	0.262	0.52	0.226
<i>C</i>	4	2	7.5	2.79	7.0	3.03	1.15	0.239	1.09	0.187
<i>C</i>	5	3	9.2	1.88	9.4	1.68	1.59	0.319	1.72	0.455
<i>C</i>	6	4	9.4	1.54	8.6	0.38	2.36	0.526	2.01	0.393

Discussion of Table II

The distribution of oil throughout the load is in general quite variable, particularly on the first oiling (loads 1, 2, and 3). There appears to be a tendency toward more uniform oiling, indicated by a decrease in the standard deviation, in loads 4, 5, and 6. This may be partially due to the levelling effect of repeated laundering and oiling, i.e., the repeated laundering tends to remove oil from the "high" areas, and the repeated oiling tends to fill in

the "low" areas. The greater uniformity of loads 4, 5, and 6 may also be partially explained by the fact that more complete souring was obtained in these loads. On the first addition of sour, the pH falls rapidly and then rises gradually as reaction with the soap adsorbed by the wool takes place. It is therefore necessary not only to use sufficient sour to hydrolyse all the soap present, but also to allow sufficient time for completion of the reaction. It is obvious that five minutes is not a sufficiently long souring time when dry sodium silicofluoride is used, since 0.37 lb. of sour per 100 lb. load reduced the pH to 4.9 in five minutes (load 2), whereas 0.90 lb. reduced the pH to only 5.5 after 10 min. (load 3).

The completeness of souring may be judged from the appearance of the souring liquor remaining in the washer at the end of operation 5, and of the residual oil bath remaining in the washer at the end of operation 6, both of which should be perfectly clear. On this basis it was concluded that souring was incomplete in loads 1 and 2, almost complete in load 3, and complete in the remaining loads.

When soap is destroyed by sour, free fatty acid is liberated and is deposited on the wool. The fatty acid content of the wool should, therefore, give some indication of the efficiency of the souring operation. There was considerably less deposition of fatty acid in the first two loads than in load 3; this confirmed the above conclusion. The increasing amounts of fatty acid observed in loads 4, 5, and 6 are due to the action of sour remaining in the wool from the previous laundering. This will be discussed more fully in a later section.

It would appear from the data given that the oil content levels off at 9 to 10%, but later work has shown that, with repeated oilings, the oil content may build up to 13 to 14% (see Table IV). The fatty acid content also builds up progressively and after four laundings and oilings shows no evidence of levelling off.

A fair degree of correlation was found between the oil content of the blankets and that of the pinned-on test-pieces. Such test-pieces could, therefore, be used to give an indication of the degree of oiling obtained on the load. It should be pointed out, however, that the analysis of a test-piece gives only the amount of oil picked up during the current oiling, and gives no indication of the amount of oil that may remain in the wool from previous oilings. The usefulness of test-pieces is thus limited unless the residual oil content can be reduced to a constant value after each laundering. This will be discussed further in the next section.

At this stage of the investigation, it was concluded that the process is satisfactory in so far as the oiling of previously unoiled wool is concerned, but that on repeated oilings there is an objectionable accumulation of both oil and fatty acid. Further work was therefore directed toward the correction of these disadvantages.

Control of Fatty Acid Content

The cause of the build up of fatty acid was investigated in the following manner. Two samples of wool were prepared: Sample *A* was taken from blankets that had been previously oiled four times, and was extracted with petroleum ether. This treatment removed oil and fatty acid, but did not remove sodium silicofluoride. Sample *B* was taken from new blankets, and was also extracted with petroleum ether. These samples were laundered with 0.3% soap solution at 100° F., rinsed in water, dried, and the fatty acid content was determined (Table III).

TABLE III
FATTY ACID CONTENT OF LAUNDERED WOOL

Sample	Fatty acid, %
<i>A</i> —Previously oiled wool	1.85
<i>B</i> —New wool	0.05

From these results it is apparent that sodium silicofluoride remaining in the wool from previous launderings causes excessive hydrolysis of soap during subsequent laundering, and is thus responsible for the high fatty acid content.

It was believed that the inclusion of an alkaline builder in the laundering formula would neutralize the excess acidity of the wool due to the presence of sour, and thus prevent further decomposition of soap. The alkali should also react with any fatty acid already deposited on the wool, so that at the conclusion of the treatment, the only fatty acid present would be that due to the decomposition of the small amount of soap left in the wool after rinsing.

Since strong alkalies have an injurious effect on wool, it was necessary to select one that would not raise appreciably the pH of the soap solution. Of the alkalies commonly used in laundering, sodium sesquicarbonate ("modified soda") was found to be most satisfactory in this respect.

Pilot Plant Trial

A pilot plant trial was carried out, using a 24 by 24 in. monel laundry wash wheel. Loads 7, 8, and 9 were made up of blankets that had been previously oiled four times, while load 10 was composed of new blankets.

Each load weighed approximately 15 lb. and was laundered according to the following formula:

Operation	Materials	Time, min.
1. Suds	Soap + modified soda	10
2. Suds	Soap + modified soda	10
3. Rinse	Water	5
4. Rinse	Water	5
5. Sour	Sodium silicofluoride	10
6. Oiling	Stock emulsion	10

The water level was maintained at the rinse level, and the temperature at 100 to 110° F. during each operation.

The water used was unsoftened Ottawa city tap water having a hardness of 4.5 to 5.0 grains of calcium carbonate per Imperial gallon. Soap flakes were used in an amount equal to 2.2% of the weight of the load. The modified soda was a mixture of 55.8% sodium carbonate and 44.2% sodium bicarbonate. The soap : soda ratio is indicated in Table IV. Dry sodium silicofluoride was added in an amount equal to 1% of the weight of the load. The stock emulsion was prepared as previously described and the volume estimated to give 5% oil on the wool was used.

At the conclusion of the oiling operation the blankets were hydro-extracted and dried at room temperature by hanging. Three consecutive launderings and oilings were given. In all of these trials complete exhaustion of the oil bath was obtained.

Samples of the oiled blankets were taken for analysis and in some cases samples were also removed immediately prior to oiling (between operations 5 and 6). The oil and fatty acid content of the samples are given in Table IV, in which each figure is an average of six determinations.

TABLE IV

EFFECT OF SOAP : ALKALI RATIO ON OIL AND FATTY ACID CONTENT OF OILED WOOL BLANKETS

Load No.	7		8		9		10	
Previous history	4 launderings and oilings		4 launderings and oilings		4 launderings and oilings		New blankets	
Ratio soap : mod. soda	5 : 1		3 : 1		1 : 1		3 : 1	
Number of launderings or oilings	Oil, %	Fatty acid, %	Oil, %	Fatty acid, %	Oil, %	Fatty acid, %	Oil, %	Fatty acid, %
Controls	10.2	1.84	10.2	1.84	10.2	1.84	0.1	0.01
1st laundering	—	—	—	—	7.8	1.54	—	—
1st oiling	12.3	3.13	12.5	3.18	10.1	1.44	4.4	0.67
2nd laundering	—	—	—	—	3.6	0.43	1.4	0.54
2nd oiling	13.4	3.78	12.0	3.51	9.1	0.38	4.8	0.49
3rd laundering	—	—	—	—	5.6	0.38	—	—
3rd oiling	13.6	4.06	12.8	3.11	10.8	0.39	5.7	0.37

Discussion

From the data presented it is apparent that, in the processing of previously unoled blankets, the inclusion in the laundering formula of soap and modified soda in a 3 : 1 ratio maintains the fatty acid content of the wool at a satisfactory level. If, however, the fatty acid content has been allowed to build up (e.g., from the use of insufficient modified soda in previous laundering), it is necessary to increase the proportion of modified soda for several launderings in order to reduce the fatty acid to the desired level.

The inclusion of modified soda also assists in the removal of oil from the wool during the laundering process, and hence tends to prevent too great a build-up of oil on the treated blankets. Harwood, Powney, and Edwards (2) have suggested that the oil content should not greatly exceed 7%. Although the data are somewhat limited, it would appear that the proposed procedure would meet this requirement satisfactorily.

One analytical figure (1.4%) was obtained for the residual oil content after laundering. It may be inferred, however, from the oil content of the treated blankets that the residual oil is reduced to 1 to 2% after each laundering. It would therefore be possible to use test-pieces to give an approximate estimation of the oil content. If the process is carried out carefully, however, there would seem to be little need for analytical control. As long as complete exhaustion of the oil bath is obtained as judged visually, it is certain that the wool will contain sufficient oil, and an excess can readily be detected by the slightly greasy feel that becomes apparent when the oil content exceeds 8 to 9%.

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AN AIR-BASE STEREOSCOPE¹

By R. H. FIELD²

Abstract

Three magnifying stereoscopes, intended for orienting stereo pairs of vertical air photographs and then ruling in the directions of the projections of the air bases on the photographs, were designed and constructed in the National Research Laboratories for the Department of Mines and Resources. Some features found useful in previously designed photogrammetric instruments were incorporated.

Introduction

In 1938 an instrument, known as the Stereograph (2), was designed and constructed for the purpose of plotting to scale the successive plan projections of the principal points in a strip of air photographs, according to the requirements of the Associate Committee on Survey Research. In addition this instrument was provided with means for the direct measurement of X and Y co-ordinates, parallax, and correspondence errors. It could also be utilized for plotting to scale the true projection of any point or outline depicted in a pair of untilted stereo air photographs.

A magnifying stereoscope was incorporated in the Stereograph, and this feature was found to make it preferable over other available instruments for the actual determination of the plan of the air base on each of successive pairs of vertical air photographs, required for the usual graphical method of radial triangulation. The need for this particular operation became so great in the Air Surveys Office, Department of Mines and Resources, that it prevented the Stereograph from being applied to the metrical and other operations for which it was intended. Accordingly it was decided to make three new instruments for the sole purpose of ruling air bases on the photographs, and which would incorporate those elements of the Stereograph found so useful for this purpose.

Principle

The new instruments were designed by the author and built by the Instrument and Model Shop from working drawings prepared in the Metrology Section. Each consists of a trough-shaped cast-iron base on which are mounted rails supporting and guiding two carriages fitted with pivoted platens to which the photographs can be clipped. Journal type ball bearings form the supporting and guiding members between carriage and rail, in the manner described in Ref. (1). Above the carriages the viewing portion of the fixed stereoscope is supported by a bracket carried from a projection at the back of the base casting.

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Two handwheels are provided. That at the left moves both carriages together along the base direction, and that at the right changes the separation of the two carriages. When the photographs have been correctly oriented, as described below, a straight-edge carried on hinged arms is pulled down so as to be on the platens while the air base projection is ruled in.



FIG. 1.

For the purpose of making this orientation the diaphragms at the focus of the eyepieces are ruled with lines, as shown at (a), Fig. 1. If these marks are adjusted by the observer to give good "fusion", when viewed against a white background, he apparently sees a single cross (b). By means of the mirror adjustment (2, p. 68) the image of each of the points marking the centres of rotation of the platens can be made to traverse the short line of each mark (a). Adjustment of the eccentric ball-bearing arbors at the rear of the carriages permits both centres of rotation to move along the same base direction, if this condition is not already fulfilled.

If, now, a pair of stereo photographs be viewed when correctly set, the cross (b) appears to touch the ground in the stereo "model". Change in the separation along the base direction (operation of the right handwheel) will cause the cross apparently to penetrate into or rise from the ground—or, in the case of a large separation, to break into its components. Movement of one photograph relatively to the other in a perpendicular direction will cause the two short lines to become non-linear, i.e., (c). If the "weight" of the diaphragm rulings be properly chosen for the average photograph, this phenomenon furnishes an extremely sensitive method for setting a stereo pair into correct fusion. For orienting air photographs on the correctly adjusted instrument by this method, the following program is used.

One photograph carriage is placed and held so that the image of the centre of rotation falls on the intersection of the mark in the appropriate side of the stereoscope. The correct photograph (say *A*) is then clipped on the platen so that its principal point is placed exactly over the centre of rotation, i.e., the image of the cross marked on the photograph is made also to coincide with the intersection of the mark at the focus of the eyepiece. The carriages are now traversed, and the operation repeated for the second photograph *B* of the stereo pair.

Keeping *B* centered, the spacing of the carriages is changed by operating the parallax handwheel until fusion is approached. Lack of correspondence is overcome by slowly rotating *A* with the aid of the tangent screw on the carriage, until the image of the detail at *B*'s principal point seen by viewing *A* is brought into the base direction of the instrument. This condition is reached

when the cross appears as (*b*). In a similar manner, with the principal point of *A* under stereoscopic observation, *B* is rotated into the correct orientation. The direction of the air base can then be ruled with the aid of a needle guided by the straight-edge.

Photograph *B* is then turned 180° in azimuth by rotating its platen, and the third photograph of the strip substituted for *A*, which permits the succeeding air base to be marked on the photographs concerned.

Description

Fig. 2 is a photograph of a completed air-base stereoscope and Fig. 3 shows the same instrument with the platens and lamp removed so that more detail can be seen.

Base.—The main casting is rigid, and is supported through three lugs, one each at the extreme ends near the front and the third at the rear centre. Turned feet are threaded into these lugs. A bracket at the rear, cast integral with the main frame, carries a ribbed casting to support the stereoscope above the photographs. The guide and supporting rails for the carriages are ground on the working faces. To facilitate grinding, these rails were each made in two pieces. The rear rail is dowelled in position while a small bracket is screwed to the front centre of the main frame to support the splice in the front rail. Ball bearings are fitted at all the journal and thrust bearings for the translation and parallax screw and shafts. These bearings are housed in brackets screwed and dowelled to faced pads on the main casting.

Photograph holders.—The photograph holders are essentially the same as those used in the Stereograph (2) except that no provision is made for correspondence settings (motion of the whole platen perpendicular to the base direction).

Translation movement.—For translating both carriages together along the base direction, the left handwheel is coupled by mitre gears to an acme thread screw, inside the main casting.

Parallax movement.—To yield stereoscopic fusion it is necessary to change the spacing of the two carriages as desired. For this purpose the right handwheel drives, through bevel gears, a square shaft behind and parallel to the translation screw. Fig. 4 is a diagrammatic sketch of the mechanism and couplings utilized in providing the necessary movements in such a manner that they can be easily operated and yet be free from backlash.

A, Fig. 4, is the translation screw, *B* the square shaft, and *C* the housing for the nut engaging with *A*. Actually this nut was made in the form of a half-nut, engaged or disengaged by means of the handle seen between the two carriages in Figs. 2 and 3. Practical experience will show whether this feature is worth while. A long sleeve, *D*, Fig. 4, with square-holed bearing portions at each end, is free to slide along *B*. For most of its length *D* is cut with a vee thread of 20 t.p.i., and engages with a long nut, *E*, which is split on one

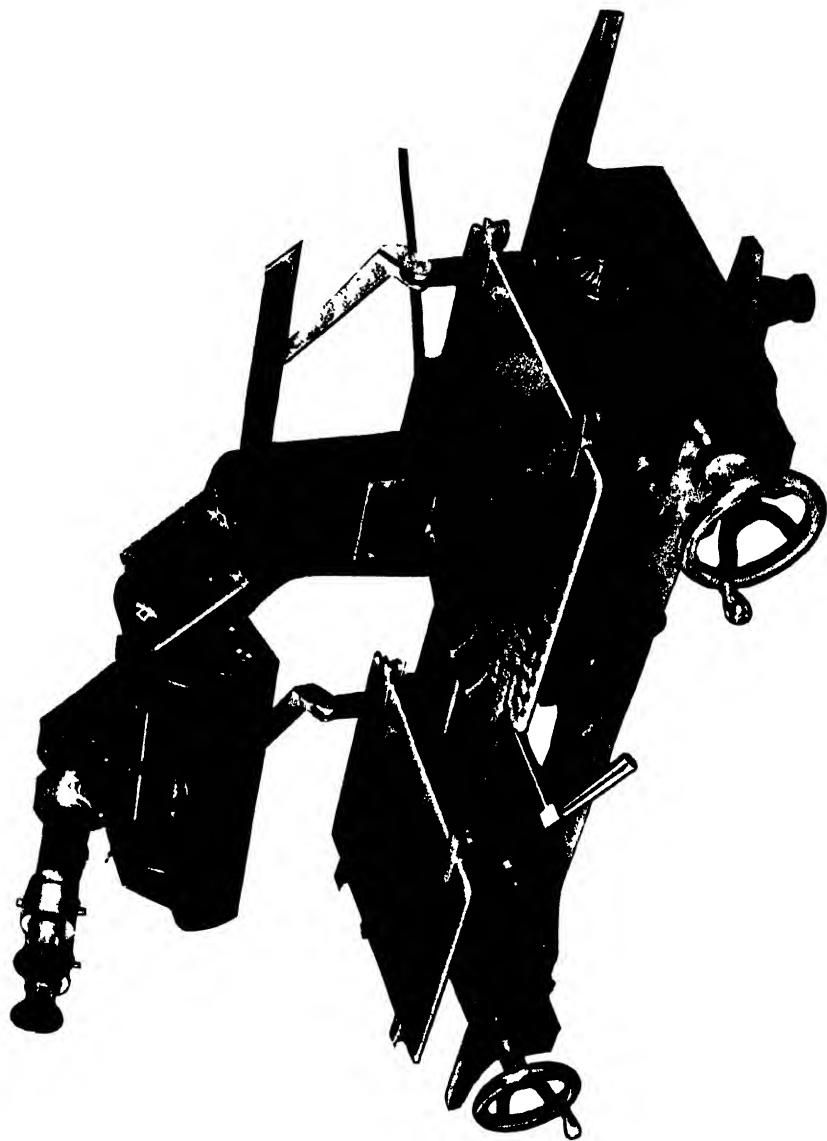


FIG. 2. .A complete air-base stereoscope.

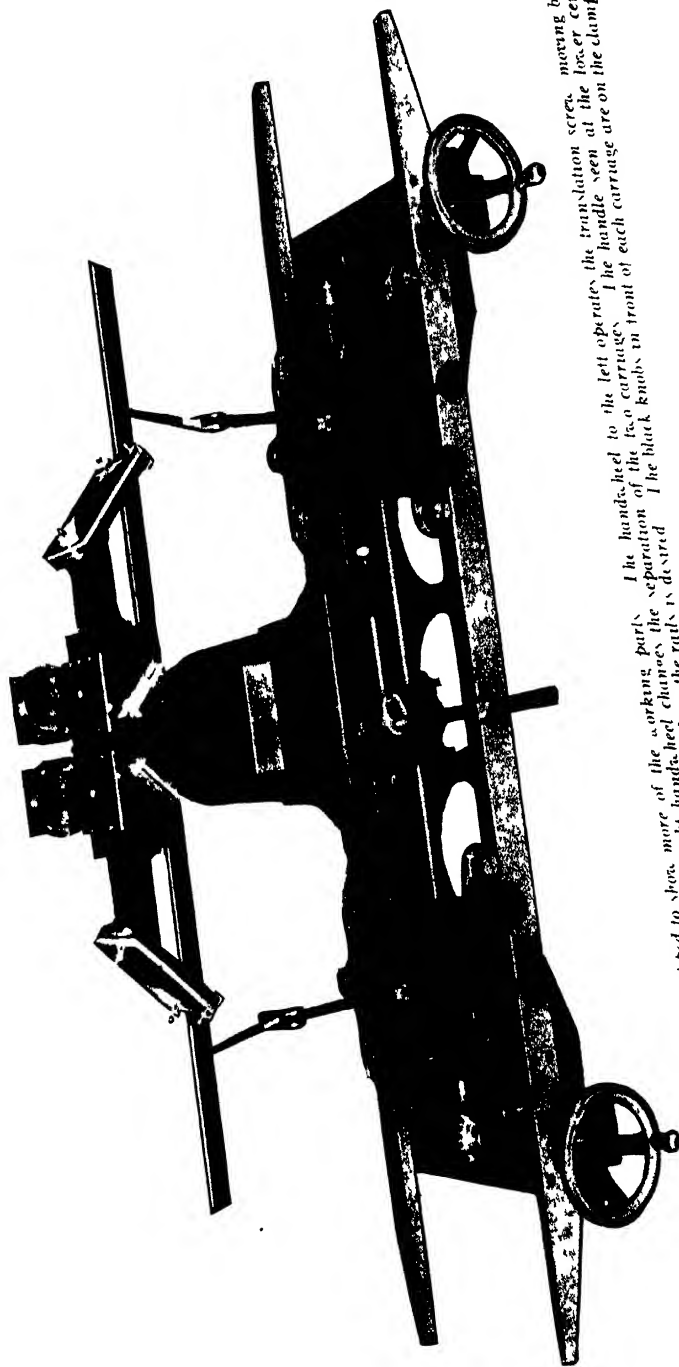


FIG. 3. The instrument partially stripped to show more of the working parts. The hand-wheel to the left operates the translation screws, moving both carriages together along the rails. Operation of the right hand-wheel changes the separation of the two carriages. The handle seen at the lower centre engages or disengages the translation nut when quick hand movement along the rails is desired. The black knobs in front of each carriage are on the clamping and slow motion tangent screws of the platen pivots.

side and fitted with screws for taking up wear. It can be seen from the diagram that rotation of *B* will cause the nut *E* to move along *D*, while the whole assembly can slide freely along *B*, under the control of *A*.

To avoid unnecessary frictional or jamming resistance both nuts are constrained only in the sense preventing rotation about the axes of their respective screws. Thus, *C* is fitted with two lugs, *F*, provided with holes engaging pins

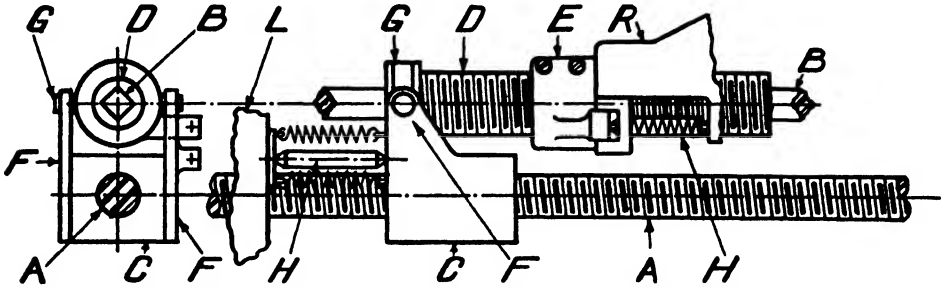


FIG. 4. Diagram to illustrate the translation and coupling mechanism for the photograph carriages.

turned on a collar, *G*, which runs in a groove on *D*, and makes contact only at the top and bottom of the cylindric portion of the groove. The rotation of nut *E* is prevented by a lug integral with it, and engaging with a groove in a plate attached to the right carriage. Pointed struts, *H*, with spring closure, are used to impart pure translation movement to the respective carriages, *R* and *L*.

The whole assembly operates very successfully. Lost motion is inappreciable, and yet the lightest finger touch on either handwheel will move the photographs.

Stereoscope.—The optical portion of the instrument is almost an exact copy of the similar part of the Stereograph (2). The elements, including front surfaced mirrors, were made entirely in the Optics Section of the Division. To facilitate viewing, a pair of small fluorescent lamps is mounted in a cover seen just beneath the mirrors in Fig. 2.

Straight-edge.—The straight-edge is attached to arms as seen in Figs. 2 and 3. Normally it is held conveniently out of the way against the stereoscope-supporting bracket, by means of a spring and ball fitted to the hinge. It can be brought down on to the photographs as desired, and nuts are provided at the base of the fixed vertical hinge arms, for use in adjusting the edge to the line of centres of the platens.

Acknowledgment

Messrs. G. C. MacKiddie and W. C. May, of the Instrument and Model Shop, built the three air-base stereoscopes here described. They introduced a number of improvements to details of the design which contributed materially

to the successful functioning of the instruments. Messrs. R. B. McKay and J. Carroll, of the Department of Mines and Resources, gave valuable advice from the operational point of view.

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A DIVIDING HEAD FOR LABORATORY USE¹

BY R. H. FIELD²

Abstract

The description is given of an easily constructed dividing head that embodies the circles and other parts of discarded theodolites. Originally designed for facilitating the inspection of munitions gauges, the instrument was found to have other valuable applications in metrology. In the appendix there is an account of the method used to calibrate the graduated circle of one of these "angle-heads", in which angles are read to single seconds of arc.

Purpose

The dividing heads here described, and called "angle-heads", were designed early in Great War II for simplifying problems presented by various types of munitions gauges where rectangular and polar co-ordinates (often in combination) have to be measured during inspection. Measurement of these gauges usually entails a more or less extended time on the part of relatively highly skilled personnel, particularly if devices of the type under consideration are not available. The angle-head was also found useful for other purposes, notably in the calibration of clinometers, and several were made during the recent war for use in the National Research Laboratories. For these instruments the graduated circles and other parts were obtained from discarded theodolites; this greatly reduced the cost and time for the construction.

First Model

The first angle-head made is illustrated in Fig. 1. The base is of cast-iron, massive, and stiffened by a web joining the two bearing housings. On the bottom are three scraped supporting pads, which, when the angle-head is assembled, have their common plane accurately parallel to the axis of the spindle. Bronze is used for the split bushings, which are hand-scraped. One bearing is of the journal type, with collars turned on the steel spindle. The tail end of the spindle is bored to fit the cone of the lower portion of an old transit, having a tolerably good horizontal circle. For the adaptation the only special parts required were a small bracket and opposing screws, to locate and adjust the vernier fitting of the transit.

The faceplate is 8 in. diam. and made of cast-iron. It was finally surfaced in the lathe after fitting on the spindle, and is secured by screws tapped into the outer collar. A series of tapped holes is distributed over the faceplate to permit the clamping of fastenings, gauges, etc., as required, and there is also a groove milled across the centre and finished so as to have its sides symmetrical with respect to the spindle axis. Two small brackets fitted with

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centres can be located in this groove, and clamped to the faceplate, for the purpose of holding, say, small taper gauges with centre-holes. The head end of the spindle is bored to receive a standard lathe centre. As a rod cannot be used to remove this centre, it is machined with a collar to facilitate extraction. The tailstock, seen to the right of the faceplate in Fig. 1, is used in conjunction with the angle-head, for indexing or measuring work carried by two centres.

A groove $3/8$ in. wide is turned in the spindle of the angle-head between the bearings, to locate the clamp. This is of bronze and split for assembly purposes. The lower half is relieved so as to contact the surface of the groove over two small areas, spaced 120° apart. The clamping shoe is made an accurate fit in a slot formed in the upper half, and is closed by means of a knurled-head screw. With these parts properly fitted, a positive clamping action, with no rotation imparted to the spindle, is attained by light finger operation of the screw.

Slow rotation of the spindle is given by a 0.375 in. \times 24 t.p.i. screw, which is bored up the centre and fitted with a round-ended strut. The outer end of the strut fits into a cupped recess at one end of a horizontal lever, pivoted to the base at the other end. Near the centre of the lever a second strut imparts movement to the tail of the clamp. Closure is maintained by a compression spring in a tube on the far side of the web in the main casting. A pin couples this spring to a recess in the clamp tail. By these means a steady slow-motion is obtained free from jerks or irregularities.

APPLICATION

Many uses for this appliance come at once to the minds of toolmakers and inspectors. Recently the theory and practice of many of these applications have been published by Barnard (1), and so need not be discussed here.

An important constant is the height of the spindle axis above the plane of the three pads, which has to be determined with the aid of a mandrel of measured diameter, by obvious methods.

Precision Model

The need for the calibration of precision clinometers and other work where an accuracy of one or two seconds of arc was necessary led to the building of a larger instrument, which is illustrated in Fig. 2. It was designed to take the 300 mm. diam. graduated circle and reading microscopes of a primary triangulation theodolite. In this case a 10 in. diam. faceplate was fitted to each end of the spindle, that at the circle end being threaded on, after the fashion of a lathe faceplate. A long tangent screw, threaded 50 t.p.i., is used, acting directly, in this case, on the tail of the clamp through a spherical-ended steel strut, bearing against a hard recessed shoe, inserted in a hole in the screw. A tension spring maintains closure, and is hooked to the middle of a horizontal lever on the far side of the central web. Pressure from the

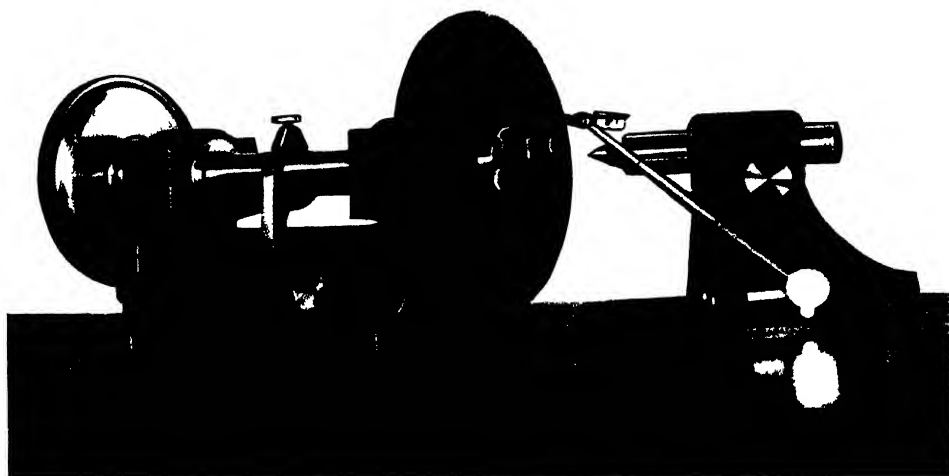


FIG. 1 *First model, angle head with circle reading to one minute*

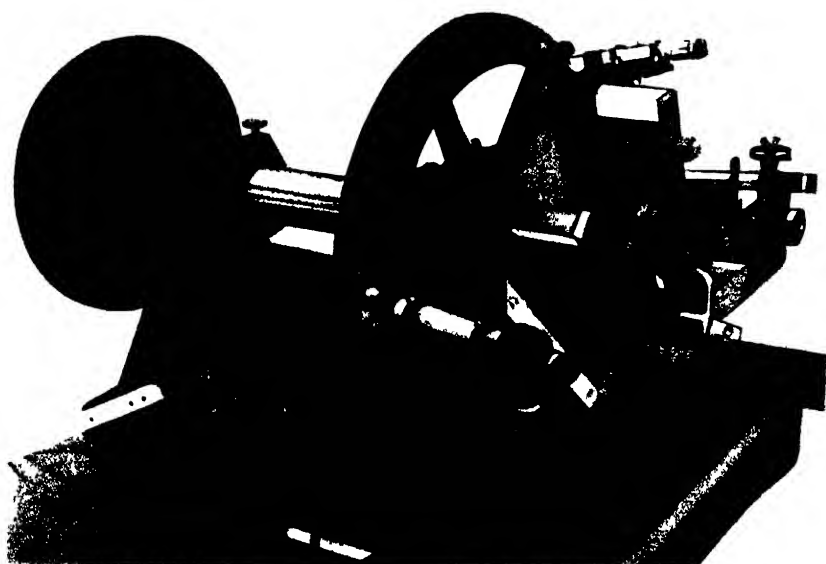


FIG. 2 *Second model, angle-head, with two faceplates and circle reading to one second*
In this view the right faceplate carries the mounting and prisms used for the calibration of the circle by the method described in the Appendix

end of the lever to the clamp tail is transmitted through a second strut, located in conical recesses in the lever-end and clamp tail.

The circle is graduated at 5' intervals, one division of the microscope drums being equivalent to 1". This circle was calibrated after the instrument was completed, by the method described in the appendix.

APPENDIX

Calibration of the Divided Circle of the Large Angle-head

PRINCIPLE

Expression for a reading.— It is shown in textbooks on astronomy, e.g., (2, Chap. II), that the general expression for the readings of the respective two *exactly* opposite verniers (or micrometer microscopes) on the alidade of a divided circle are:

$$A = Z - e \sin (Z + E) + \epsilon_z \quad (1)$$

and

$$B = 180^\circ + Z - e \sin (180^\circ + Z + E) + \epsilon_{180^\circ + z}, \quad (2)$$

where Z = The reading if the alidade were exactly centered with respect to the circle and if the graduations were correctly placed;

e = The eccentricity, i.e., the angle subtended by the line joining the centre of the circle and the axis of rotation of the alidade, at the circumference of the circle;

E = The azimuth (on the circle) of the line just mentioned;

ϵ_z = The error in the position of the graduation at the nominal angle, Z , on the circle.

Eccentricity.— Hence the mean reading $(A + B)/2$ is independent of the errors due to eccentricity, and involves the mean of the two opposite graduation errors. It would also appear from the theory that a variable eccentricity, e.g., such as would be introduced by a departure of the alidade pivot from true cylindrical or conical form, or by looseness in the fit of that pivot, would have no effect on the mean of the two readings. That is, provided this fit is not too tight when, as has been shown by Rannie (3), important errors are introduced; the degree of perfection in the fit of the alidade pivot may not be of first order importance provided both verniers are read at each observation. It also follows from Equations 1 and 2 that in calibrating a circle having opposite verniers, the observations can be limited to an arc of 180° . In certain cases, however, as with some of the observations described below, the calibration test is carried right around the circle for check purposes. The values of E and e can be obtained from the readings made in the course of the tests described below or, as was actually done in this case, in a special test, from reading the two micrometers at a number of points distributed around the circle. Actually E and e have only to be known if subsequently angles are to be measured by means of a single vernier. They also are required

if the value of ϵ is to be determined throughout the 360° , e.g., to assess the degree of perfection attained in the circle-graduating operation.

Graduation errors.—A circle graduated throughout its circumference contains by definition exactly 360° , beginning and ending at any single graduation mark. Therefore, one of the main difficulties in many types of instrument calibration—the comparison with an accepted reference standard—is overcome. It is merely necessary to find some constant external reference angle that is nearly an exact submultiple, say $1/n$, of, first 360° and subsequently, of the subdivisions already calibrated, and to compare this reference angle successively with each of the n equal parts of the circle or interval under study. By suitably drawing up the test program, it is generally necessary that the reference angle remain constant for only a short time. As a further refinement, by making the n observations first in the one direction and immediately afterwards in the reverse order, it can usually be assumed, without serious error, that any small change in the reference angle has been taking place at a uniform rate during the observations, and so is eliminated in the means.

COMPUTATION

Using the notation of Equations 1 and 2, we find the expression for the micrometer readings at the two points subtending an angle $Z_1 - Z = \phi$ on the circle to be:

$$A_{z_1} + B_{z_1} - A_z - B_z - \epsilon_{z_1} + \epsilon_z - \epsilon_{180^\circ + z_1} + \epsilon_{180^\circ + z} = 2\phi. \quad (3)$$

Considering the diametral mean reading, R_z , and the diametral mean error, ϵ'_z , for a given pair of observations, Equation 3 becomes:

$$R_{z_1} - R_z - \epsilon'_{z_1} + \epsilon'_z = \phi. \quad (4)$$

In the actual tests a series of n such pairs of observations is made, and the sum of the n equations, 4, is:

$$R_{n\phi} - R_0 - \epsilon'_{n\phi} + \epsilon'_0 = n\phi. \quad (5)$$

In the first test $n\phi$ is chosen so as to be nearly 360° , and hence $\epsilon'_{n\phi} = \epsilon'_0$ and

$$\phi = (R_{n\phi} - R_0)/n \quad (6)$$

Inserting this value of ϕ in the first of the set of Equations 4, the value of ϵ'_ϕ is determined, and this value permits the value of $\epsilon'_{2\phi}$ in the second equation to be found and so on. Actually it is not necessary that the initial reading in each measurement of ϕ coincide exactly with the final reading of the preceding measurement, and ϕ can be obtained from the mean of the n measurements. A very simple tabulation suffices for the computation as shown in the example (Table I). The process can be extended down to the smallest graduation interval on the circle.

The values of E and e , as well as the small angle, α , by which the spacing of the fiducial points of the micrometer microscopes differs from 180° (Equations 1 and 2), can be found (2) from reading the two micrometers at a suffi-

TABLE I
CIRCLE CALIBRATION
TYPICAL SET OF OBSERVATIONS, AND REDUCTION

Observations				Mn	Mn F and B	Δ	$\Sigma (\Delta)$	* Interval correc- tion	Diametral correction	
Microscope A		Microscope B								
Reading	Diff.	Reading	Diff.							
0° 01' 32"	"	180° 02' 20"	"	"	"	"	"	"	"	"
3 01 49	17	183 02 34	14	15.5	16.2	-1.6	-1.6	+0.6	-1.0	(3°)
3 01 45		183 02 15								
6 01 30	15	186 02 28	13	14.0	14.5	+0.1	-1.5	+1.2	-0.3	(6°)
6 01 30		186 02 14								
9 01 45	15	189 02 27	13	14.0	13.2	+1.4	-0.1	+1.8	+1.7	(9°)
9 00 51		189 01 33								
12 01 05	14	192 01 47	14	14.0	14.0	+0.6	+0.5	+2.4	+2.9	(12°)
12 00 44		192 01 26								
15 00 59	15	195 01 40	14	14.5	15.2	-0.6	-0.1	+3.0	+3.0	(15°)
				Mean, $\phi = 3^{\circ}00' 14''.6$						
15 01 08		195 01 48				*From previous tests:— Correction at 0° = 0'' Correction at 15° = +3''.0 3''.0 \div 5 = 0''.6				
12 00 51	17	192 01 33	15	16.0						
12 00 39		192 01 20								
9 00 24	15	189 01 07	13	14.0						
9 01 42		189 02 26								
6 01 29	13	186 02 14	12	12.5						
6 01 14		186 01 59								
3 00 58	16	183 01 45	14	15.0						
3 01 55		183 02 42								
0 01 36	19	180 02 27	15	17.0						

ciently large number of points around the circle to compensate for the effect of the graduation errors. Values can then be calculated for the individual residual errors ϵ , in Equations 1 and 2, after substituting the now known quantities.

APPARATUS

The reference angles, ϕ , were obtained by mounting two prisms with aluminized working faces on the angle-head, setting the faces at the desired angle to one another and using an autocollimator to bring the faces successively into the same position, i.e., normal to the axis of the fixed collimator.

The photograph of the precision angle-head, Fig. 2, actually shows the prisms and auxiliary apparatus mounted in position for part of the calibration test. This test was carried out with the aid of the regular collimator system of the Metrology Laboratory. In this apparatus the collimators are sup-

ported on massive concrete piers, resting on the underlying rock, in an air-conditioned room below ground level. No appreciable change in the direction of the collimators can be detected over extended periods, so they serve excellently as fixed reference directions in tests of this kind. A reflecting eye-piece was fitted to the horizontal collimator utilized in this particular case. The angle-head was set up on a levelling base placed on the regular testing stand, at the centre of the collimator system, in order that its spindle could be made horizontal and placed approximately normal to, and at the same height as, the axis of the collimator.

It was necessary so to mount the prisms that they could be readily adjusted with their faces parallel to the spindle axis of the angle-head and could be turned around as a unit after the measurement of each angle ϕ . For this purpose the tribrach and horizontal limb of a discarded small mountain transit was mounted on the faceplate, as seen in Fig. 2. At first some irregularities were found in the readings, but these were traced to looseness in the conical centres of the old tribrach. They were overcome by fitting a nut that permitted the cones to be drawn tight after the setting of the prism assembly at the beginning of each of the n measurements.

In the actual test one observer set the initial position of the prism assembly nearly correct, under the command of a second observer at the collimator eye-piece. He then clamped the cones of the small tribrach, and observer No. 2, with the aid of a wooden rod temporarily clamped to the head of the angle-head tangent screw, brought into coincidence the collimator cross wires and the image of the wires reflected from the first prism face. Observer No. 1 then read both angle-head micrometers. He next unclamped the angle-head, rotated the faceplate through, approximately, the angle ϕ , clamped it, and observer No. 2 brought on to the cross wires the image reflected from the second prism face. Finally, observer No. 1 again read the two micrometer microscopes. This program permitted the test to be carried out quite rapidly.

A typical example of the readings and their reduction for the interval 0° to 15° is given in Table I.

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NOTES ON THE TESTING OF SEXTANTS¹

BY G. O. WEST²

Abstract

In World War II a shortage of mariners' sextants resulted in the collection by the Royal Canadian Navy of used sextants from various sources. These were, as far as possible, reconditioned and verified by the National Research Laboratories, Ottawa. For this work, and for calibrating new sextants, some modifications were made to the collimator system regularly employed for the verification of angle-measuring instruments. A brief description is given of the apparatus and of the procedure developed for testing sextants, together with a few remarks on sextant errors.

Introduction

During the recent war, there was an urgent demand for mariners' sextants, and the Royal Canadian Navy collected a number of second-hand instruments, which were reconditioned and calibrated at the National Research Laboratories. Also, during the war a Montreal firm designed and constructed a dividing engine for cutting the arcs of sextants, and consequently was able to undertake the manufacture of new instruments. Representatives of the firm came to the Laboratories from time to time for advice, and some of their sextants (the first, so far as is known, to be built in Canada) were subsequently submitted to the Laboratories for certification.

Only a few mariners' sextants had been calibrated prior to the war. These were verified for the accuracy of their angular indications by measuring with the sextants the angles subtended by church spires, chimneys, and other suitable distant objects, visible from the roof of the Laboratories, the angular distances between these various targets having first been determined by means of a theodolite. Under the new conditions imposed by the war, it became imperative to devise an indoor test for greater speed and accuracy, and for independence from weather conditions.

This was done by additions to the collimator system already in use for testing transits, levels, and other optical measuring instruments. This apparatus, which is now installed in the National Research Laboratories, was devised originally about 1910 by the well known former Surveyor-General of Canada, Dr. E. G. Deville (1).

The theory of the sextant is set out in textbooks on astronomy and surveying (2), and it is there shown that instrumental errors may be due to defects in:—

1. The perpendicularity of the index mirror to the plane of the arc,
2. The perpendicularity of the index axis to the plane of the arc,
3. The parallelism of the line of collimation of the telescope to the plane of the arc,

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² Technical Officer.

4. The perpendicularity of the horizon glass to the plane of the arc,
5. The graduations on the arc (or, in the case of modern sextants, the spacing of the sector teeth),
6. The optical characteristics of the mirrors and shades.

A slight modification of the usual collimator set-up as employed for the calibration of most other instruments for measuring angles is required in the case of sextants, owing to the fact that the two direction lines (which determine the angle under observation) do not intersect at the geometric centre of the measuring arc.

At the National Physical Laboratory in England, sextant tests are carried out with the aid of a set of collimators mounted in pairs (the two units of a pair being parallel) on a hollow cast-iron arc through which water is circulated to maintain a constant temperature. As a simpler alternative, it was decided in the present case to make single collimators radiate from the centre of the testing platform, but to support the sextant horizontally on the platform in such a way that it could readily be moved, so that the axes of the two collimators under observation at a given time could be made to intersect the centres of the respective sextant mirrors. Thus the use of duplicate collimators was avoided—the sextant being recentred for each angle. This adjustment is readily made by eye to a precision that does not affect the accuracy of the angles for properly focused collimators.

Apparatus

The collimators, Fig. 1, which are located in an air-conditioned room in the sub-basement of the Laboratories, are set on concrete piers isolated from the floor and resting on the bed rock. The cast-iron supports in which the telescopes are mounted are each carried by three foot-screws. Discarded surveying instruments were utilized in making the additional collimators needed for verifying sextants.

The special table supporting the sextants to be tested is rigidly attached to the top of the vertical axis of an old triangulation theodolite base, visible in Fig. 1. This assembly rests on the regular testing platform, which is at the intersection of the collimator axes, and is adjustable for height. The sextant table can be adjusted for horizontality by means of the foot-screws of the theodolite base and can also be turned in azimuth and set in any required orientation by the tangent screw assembly.

Fig. 2 is a plan diagram of the collimator arrangement that is seen in Fig. 1.

The angular distances between the various collimators that are used to determine the errors of graduation of a sextant are approximately as follows:—

<i>E</i> to <i>D</i>	15°	<i>II</i> to <i>F</i>	75°
<i>F</i> to <i>E</i>	30°	<i>F</i> to <i>A</i>	90°
<i>F</i> to <i>D</i>	45°	<i>H</i> to <i>E</i>	105°
<i>A</i> to <i>E</i>	60°	<i>II</i> to <i>D</i>	120°



FIG. 1 Collimator system for testing sextants. The larger collimators seen were already in use for other purposes. Additional smaller collimators were made from discarded surveying instruments. A modern sextant is shown under test. On the pier is an old wooden octant submitted to the Navy during the war.

Collimator *G* is used only in testing the shades, and collimator *C*, which has a large objective, is employed for setting the zero position of the vernier or drum.

For targets, thin metal diaphragms with slots were first tried in the new collimators, but better results were obtained with graticules carrying cross

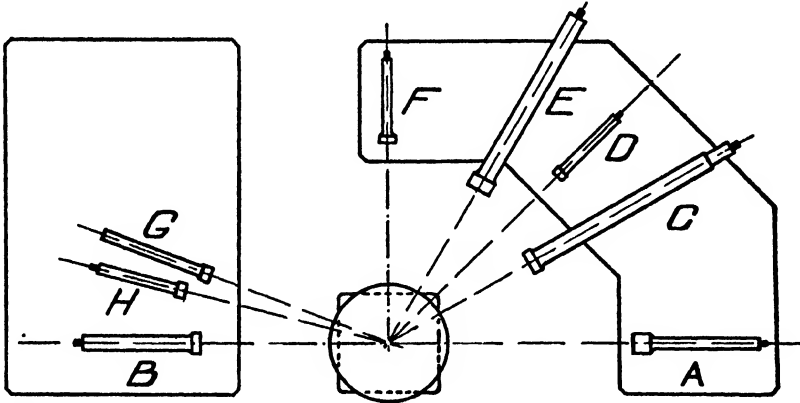


FIG. 2. Diagrammatic plan of the collimator system.

lines of fine tungsten (lamp filament) wire. When measuring a given angle the arrangement permits the single vertical wire in the image from one collimator to be set midway between the pair of wires in the image from the second collimator.

Collimator *E* is fitted with a special glass diaphragm on which, in addition to a pair of vertical lines, seven nearly equidistant vertical lines are ruled. This glass diaphragm was made specially for the purpose by the Optics Section. These seven lines are spaced at 10' intervals and are used for measuring the progressive errors of micrometer screws.

All the cross wires are illuminated by 10-v. lamps with ground glass diffusers. The intensity of each light can be changed by means of a variable rheostat in a control panel adjacent to the observer and visible in Fig. 1.

Test Program

The following test program was adopted, based in part on classical methods, but with some modifications that are thought to be original.

(a) Preliminary Examination

The sextant is first examined for obvious defects. Some of the major faults that are encountered include, for example: lack of coincidence in the graduations of the vernier and arc; backlash in the tangent screw assembly; parallax in reading the scale in the case of graduated instruments; and incorrect mating of the cone worm with the sector in micrometer sextants.

(b) Perpendicularity of the Index Mirror and Pivot

To check these errors, the sextant, with the telescope assembly removed, is set up on the levelling table with the index mirror between collimators

A and *B*, whose optical axes are coincident and truly horizontal. An auto-collimating eyepiece, replacing the lamp, is fitted to each of the collimators.

First the sextant is adjusted so that the horizontal cross wires of *B* and their reflected image from the index mirror, as seen by an observer looking through the eyepiece of *B*, are coincident. In this position the index mirror is truly vertical. The index arm is then rotated 180° in azimuth. If the horizontal cross wires and their reflected image, as seen by an observer looking through the eyepiece of *A*, do not coincide, half the discrepancy is taken up by the foot-screws of the theodolite base and half by the adjusting screws on the frame of the index mirror.

When this adjustment is completed the plane of the index mirror and the pivot (unless the pivot is distorted) will be truly vertical when the mirror is at right angles to the line of collimation of the telescopes.

The perpendicularity of the index axis to the plane of the arc can now be tested. This can quickly be done with the aid of a small spirit level laid on the graduated surface of the arc, at the same time keeping the index mirror truly vertical for any desired position of the sextant, by adjusting the foot-screws of the theodolite base and observing through the reflecting eyepiece of one of the collimators *A* or *B*.

(c) *Parallelism of the Telescope*

With the telescope assembly restored to the instrument, the telescope is directed on collimator *B*, the graduated surface of the arc of the sextant being brought truly horizontal with the aid of the small spirit level. If the line of collimation of the telescope is parallel to the plane of the arc, the image of the cross wires of *B*, when viewed through the telescope, will be in the centre of the field of view. Any necessary adjustment is made by means of the two adjusting screws on the ring carrying the telescope.

(d) *Perpendicularity of the Horizon Glass*

The sextant is directed on collimator *C*. This collimator has an aperture of 80 mm. and is therefore large enough to include parallel rays to both the index mirror and horizon glass of an ordinary sextant. The vernier being set to zero, the horizon glass is then adjusted to bring the images of the horizontal and vertical cross wires into coincidence.

(e) *Graduations on the Arc*

When checking sextant indications for the angular distances between various collimators, a telescope of $\times 10$ magnification, with adapters to fit different types of sextants, is used so as to avoid eyestrain. The angles between the collimators remain constant for several hours and can be measured very quickly, when required, by means of a Wild theodolite reading to single seconds, and which can be set up on the regular testing stand.

(f) *Mirrors and Shades*

A complete description of the effect of errors introduced by prismatic mirrors is given in Reference (2, p. 83). In the laboratories the back-surfaced

mirrors are tested individually to determine whether their faces are truly parallel. The lamp for collimator *B* is replaced by a Ramsden eyepiece. The sextant is adjusted on the levelling table so that each mirror in turn is placed at the intersection of the optical axis of *B* and that of one of the collimators with a relatively large angular distance from *B*, and so that the image of the cross wires can be seen by an observer looking through the eyepiece of *B*. If a mirror is prismatic a ghost image of the cross wires will also be seen. The method adopted for determining the prismatic errors of the shades is practically the same as that employed by the National Physical Laboratory. The angular distance between collimator *G*, which carries a thin metal diaphragm with a pair of vertical slits to admit the light, and collimator *B*, which has a small illuminated hole in the comb of the diaphragm, is measured by the sextant without the use of shades. Each shade is then separately introduced. Any prismatic error is measured directly by the vernier (or drum) of the sextant. Brighter lights are of necessity used on these two collimators for this purpose, the intensity of the light depending on the density of the shade being tested. For very dense shades a 900 w. condensed filament light may be necessary.

If the faces of the mirrors are not truly plane the definition of the image of the cross wires, when viewed through the high power telescope, will be distorted. In cases where such distortion is suspected the actual flatness of the mirrors is investigated by interferometric means.

(g) *Progressive Errors in Micrometer Screws*

The angular distances between the seven extra vertical lines in collimator *E* and the pair of vertical lines in collimator *F* are measured by the sextant. The progressive error of the micrometer screw is determined by comparing the sextant indications of the successive increments, which give a complete turn of the screw at 10' intervals, with the known values which were originally measured with the Wild theodolite.

Some Remarks on the Second-hand Instruments Submitted by the Navy

Some of the second-hand instruments received from the Navy were found to be considerably damaged, probably as the result of being dropped, and these presented some problems for the instrument-maker. A sextant with a bent arc or bent pivot socket is difficult to restore to its original condition. The makers usually graduate the arc after the pivot axis has been fitted.

The mirrors from these used instruments were, when necessary, resilvered. A few of them, which gave poor images of the cross wires, were found on interferometric examination to be plane. The trouble was traced to their not being properly supported in the mirror frames. If the spring clips holding the mirror in place are not directly over the studs supporting the mirror, even light pressure of the adjusting screw may cause distortion. One mirror was found on examination to be of ordinary window glass.

These second-hand instruments were an interesting assortment. One modern German sextant fitted with a micrometer drum was found to be in excellent condition and to have negligible corrections for both the angular indications and the shades. This instrument proved to be the best sextant ever tested here. The graduations on the arc of another sextant had obviously been stoned off and then restored with the aid of a pocket knife or similar instrument. On still another instrument, initials had been carved on the arc in such a way as to obliterate some of the graduations. Also included was an octant such as was used by mariners in bygone days. This instrument, of English manufacture, with an ebony frame and an ivory scale graduated by hand, is visible on the pier in Fig. 1.

Acknowledgment

The extra collimators and the levelling table for supporting the sextants were constructed by Mr. A. A. Barks of the Division of Physics and Electrical Engineering. His assistance and advice are gratefully acknowledged.

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PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

XI. EVALUATION OF *LEVO*-2,3-BUTANEDIOL AS A NON-VOLATILE ANTIFREEZE COMPOUND¹

BY K. A. CLENDENNING²

Abstract

levo-2,3-Butanediol is more effective than glycerol and less effective than ethylene glycol as a freezing point depressant for water. The considerable discrepancy that is reported between observed freezing points and values calculated from Raoult's law for solutions of these chemicals is attributed to hydration. The antifreeze property of *levo*-2,3-butanediol is not impaired by prolonged refluxing or by use in automobile cooling systems. The viscosity of 50 to 60% solutions is considerably greater than that of 50 to 60% ethylene glycol and is slightly greater than that of 50 to 60% glycerol at 20° C., the differences in viscosity between these solutions being magnified by low temperatures. Kinematic viscosity data are presented for *levo*-2,3-butanediol solutions at concentration intervals of 10% over the greater part of the liquid range.

The comparatively low surface tension of *levo*-2,3-butanediol solutions indicates a possible need for precautionary measures against creeping and foaming. Metallic corrosion is not greater than with water, barring excessive contamination with acetates. *levo*-2,3-Butanediol and ethylene glycol are judged equally satisfactory with respect to heat capacity, flash point, expansion on solidification and heating, and effects on metal finishes and rubber. Density of *levo*-2,3-butanediol solutions cannot be used as a measure of freezing point protection. The mixtures of 2,3-butanediol isomers obtained with *Aerobacter aerogenes*, *Aeromonas hydrophila*, and *Bacillus subtilis* are lacking in antifreeze properties because of their high content of the *meso*-isomer.

Introduction

The usefulness of 2,3-butanediol as a potential source of synthetic rubber prompted a vast amount of research on its production by bacterial fermentation, as well as on its purification, esterification, and pyrolysis to butadiene. Experimentation was extended to pilot plant studies at several laboratories, and commercial developments in all likelihood would have followed had other sources of synthetic rubber proved inadequate.

Several patents have been granted in which some mention is made of the usefulness of butanediol as an antifreeze (11, 14, 28). Information was not provided at the time of their issue, however, on the relative usefulness of the eight different butanediol isomers that are of interest in this application. Michael and Hopkins (20) have indicated that 1,3-butanediol is unsuitable because of the extremely high viscosity of its solutions at low temperatures. The 2,3-butanediol that is produced by *Aerobacter aerogenes* (4), *Aeromonas hydrophila* (30), and *Bacillus subtilis* (23) consists of a mixture of isomers in which the *meso*-form predominates. The diol produced by *Aerobacillus polymyxa* consists entirely of the levorotatory isomer (22, 34). Chadderton

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and Cook* observed that *levo*-2,3-butanediol has valuable antifreeze characteristics, and that these are lacking in the corresponding *meso*-isomer. Reference has also been made to the differences in antifreeze properties of 2,3-butanediol isomers by Ward, Pettijohn, Lockwood, and Coghill (34).

The popularity of an antifreeze is largely decided by the yearly expense that its use entails. Since *levo*-2,3-butanediol combines the advantage of permanence shown by ethylene glycol with a potentially low selling price, its possibilities in the antifreeze field deserve attention. The present paper reports a study of those properties of its aqueous solutions that have greatest bearing upon usefulness and performance in automobile cooling systems.

Materials and Methods

The *levo*-2,3-butanediol samples were prepared from fermented wheat mash by methods outlined elsewhere (22, 27). The mixed isomers ($[\alpha]_D^{25} = +0.8^\circ$) obtained with *Aerobacter aerogenes* consisted of the *meso*-form and 5 to 10% of the *dextro*-isomer. The 2,3-butanediol samples obtained with *Aeromonas hydrophila* and *Bacillus subtilis* had $[\alpha]_D^{25}$ values of -1.25° and -4.86° respectively. The water content of *levo*-2,3-butanediol samples was determined by reference to tables of refractometric and polarimetric data (5). Degree of contamination with esterified acetic acid was determined by a method based on saponification and back titration (21). Ethylene glycol was purified by fractional distillation *in vacuo*, the fraction selected for use showing a refractive index of 1.4320 at 25°C . Glycerol of c.p. grade was used as received, its water content being determined by measurement of the refractive index. Absolute ethanol and synthetic methanol were redistilled at atmospheric pressure before use. All solutions were made up by accurate weighing.

The freezing points were determined by the conventional procedure of supercooling the solutions, seeding with a few crystals of the appropriate component, and following the resulting temperature change by thermocouple. It was found advantageous to run a series of determinations on different samples of the same solution, seeding each at the apparent freezing point of the previous sample, the values as here recorded referring to the highest temperature at which incipient crystallization could be induced in this manner. An auxiliary unit similar to the freezing point apparatus described by Cummings (7) was used in confirming freezing point values by visual inspection. The accuracy of the determinations is considered to be of the order of 0.2°C . except at the lowest temperatures, where the possible error is judged to be 1° to 2°C . The temperature at which *levo*-2,3-butanediol solutions become solid is several degrees below that at which incipient crystallization occurs on seeding; e.g., the 50% solution, which shows a freezing point of -29.6°C ., reaches the consistency of moist taffy at -36° to -37°C . This property and the pronounced supercooling tendency of diol solutions is judged to offer a con-

* Unpublished report, December, 1942.

siderable margin of safety against solidification at the recorded freezing temperatures.

The viscosity measurements were made by the conventional kinematic method (1), using Ostwald viscosimeters immersed in constant temperature baths. Absolute viscosity was calculated by the use of specific gravity data reported elsewhere (5). Boiling points were determined in an apparatus equipped with a Cottrel pump and N.B.S. certified thermometer. Flash point was estimated by the A.S.T.M. open cup method (1). Surface tension measurements were made at 25° C. with a Du Nuoy interfacial tensiometer. Corrosiveness was assessed by the changes in weight and appearance of metal test plates after two months' immersion at room temperature. Solvent action on automobile finishes was tested by immersing variously coated steel rods in the antifreeze compounds and solutions, and observing the condition of the protective finishes after 90 min. exposure at room temperature. Effects on rubber hose connections were determined by immersing rubber samples in the antifreeze solutions for three days at 80° C. and noting the changes in physical properties by conventional objective methods.

Results and Discussion

In its major features, the freezing point curve for *levo*-2,3-butanediol in water (Fig. 1) corresponds closely to similarly expressed data for ethylene glycol and glycerol. As Table I shows, *levo*-2,3-butanediol is intermediate

TABLE I

FREEZING POINT DATA FOR METHANOL, ETHANOL, ETHYLENE GLYCOL, GLYCEROL, AND *levo*-2,3-BUTANEDIOL SOLUTIONS

Solute by weight, %	Methanol		Ethanol		Ethylene glycol		Glycerol		<i>levo</i> -2,3-Butanediol	
	F.p. observed, °C.	F.p.* calculated, °C.	F.p. observed, °C.	F.p.* calculated, °C.	F.p. observed, °C.	F.p.* calculated, °C.	F.p.** observed, °C.	F.p.* calculated, °C.	F.p. observed, °C.	F.p.* calculated, °C.
10	- 6.3	- 6.46	- 4.5	- 4.49	- 3.6	- 3.33	- 2.0	- 2.25	- 3.1	- 2.30
20	-15.3	-14.5	-10.5	-10.1	- 8.3	- 8.27	- 5.2	- 5.05	- 7.1	- 5.17
30	-26.3	-24.9	-20.0	-17.3	-14.7	-12.9	- 9.9	- 8.67	-12.4	- 8.85
40	-39.7	-38.8	-29.4	-27.0	-23.5	-20.0	-15.9	-12.0	-19.4	-14.3
50	-55.2	-58.1	-37.0	-40.4	-35.0	-30.0	-24.6	-20.2	-29.6	-20.7
60			-43.8	-60.7	-50	-45.0	-37.9	-30.3	-40.4	-31.0

* $\Delta = \frac{K}{g} \frac{100}{n}$, where Δ = F.p. lowering, K = f.p. lowering for 1 mole solute in 100 gm. solvent (18.6° C.), n = number of moles solute, g = grams solvent.

** Data of J. A. Spangler and E. C. H. Davis. *Ind. Eng. Chem., Anal. Ed.* 15 : 96-99. 1943.

between these two chemicals as a freezing point depressant for water, while ethanol and methanol depress the freezing point to a greater extent at equivalent percentages by weight.

Antifreeze solutions represent an application of freezing point lowering by solute molecules that has considerable practical interest. The organic anti-

freeze chemicals of commerce are polar non-electrolytes possessing one or more hydroxyl groupings. Information on their antifreeze properties is still highly empirical, and the relative usefulness of any new substance that is

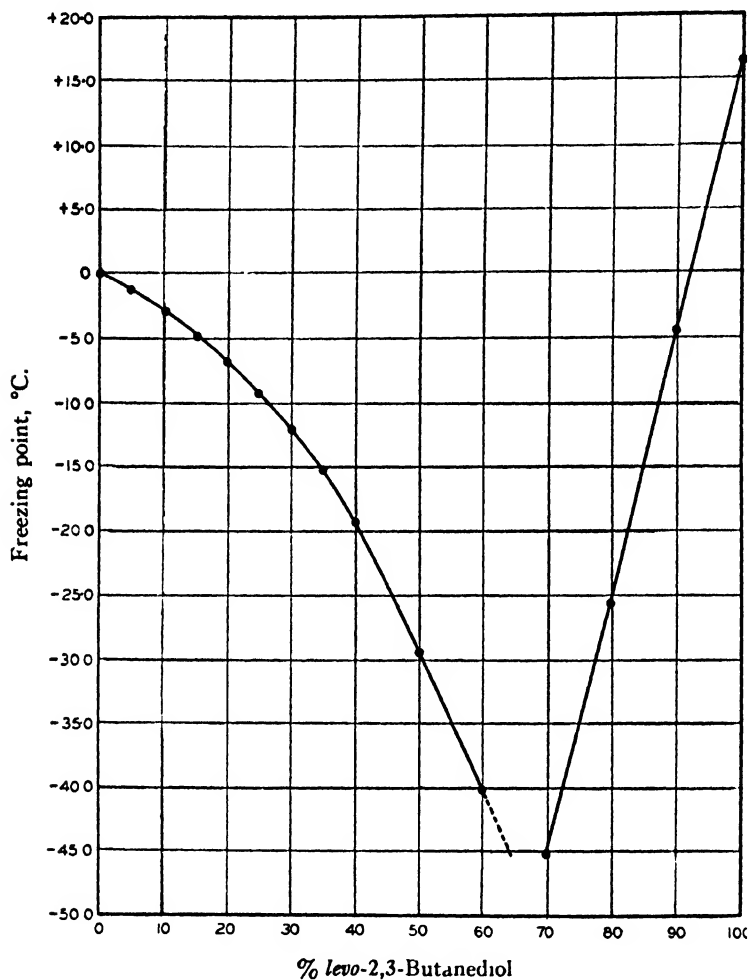


FIG. 1. Freezing points of aqueous levo-2,3-butanediol solutions.

proposed for this purpose cannot be predicted with any degree of accuracy. Raoult's law is known to apply only to "ideal" solutions, and divergences are to be expected in radiator solutions of low freezing point. Theoretical freezing point values calculated by its application to solutions of the commercial antifreeze compounds are presented in Table I, however, since they shed some light on the relative importance of molar freezing point lowering and contributing factors such as hydration.

Among the five antifreeze chemicals listed in Table I, a general relation exists between molecular weight and observed freezing point lowering;

methanol is the most effective and glycerol is the least at equivalent percentages by weight, and at most concentrations the remaining compounds take intermediate positions in the expected order. Between chemicals, the observed differences in freezing point are much smaller than would be expected from their molecular weights. Thus at 50% by weight, the calculated freezing point values for ethanol, ethylene glycol, and *levo*-2,3-butanediol are -40.4° , -30.0° , and -20.7° C., respectively, whereas the freezing point values actually were -37° , -35° , and -29.6° C. The freezing point lowering observed with *levo*-2,3-butanediol is approximately $4/3$ the calculated value over the range 0 to 60% diol, and the same tendency is consistently shown by ethylene glycol and glycerol although to a lesser extent. Up to concentrations of 40%, the observed values for ethanol and methanol also exceed the calculated values slightly, but, at concentrations of 50% and higher, the observed freezing point depression is less than would be expected from similar calculations.

Jones and co-workers (16, 17) found that the hygroscopic salts crystallizing with the greatest amount of water produced the greatest molecular lowering of the freezing point, and they indicated the importance of hydration in many instances in which freezing point lowering was exceptionally great. Hydration of the solute is evidently responsible for the abnormally large depression of the freezing point of water by ethylene glycol, glycerol, and *levo*-2,3-butanediol, all three of which are strongly hygroscopic: part of the water is bound by residual valence forces to the polar solute, the hydrated molecules then acting as units in depressing the freezing point of the water that remains. Commercial antifreeze solutions and the cellular contents of frost- and drought-resistant plants evidently have more in common than has hitherto been realized. The present indication of the importance of hydration in the freezing point lowering shown by *levo*-2,3-butanediol is supported by viscosity data presented in this paper, as well as by densimetric and polarimetric observations that will be published elsewhere (5).

Existing information, which need not be reviewed here, indicates that hydration occurs in aqueous solutions of methyl and ethyl alcohols. The question arises as to why the freezing point lowering is not strongly promoted by hydration when these two chemicals are employed. At concentrations of 40% and lower, one molecule or less of water is bound by each alcohol molecule (16), i.e., the hydration tendency is much less marked than with ethylene glycol, glycerol, and *levo*-2,3-butanediol. Pairing of solute molecules may also be partly responsible for the fact that freezing point lowering with these two chemicals is not greater than it is (16). With alcohol concentrations of 50% and higher, the solid phase is evidently an alcohol hydrate, the freezing point being progressively lowered with rising alcohol concentration as a result of hydrate solubilization.

The relatively high freezing temperature of essentially pure *levo*-2,3-butanediol has practical significance. The freezing point of pure ethylene glycol is nearly 30° lower than that of *levo*-2,3-butanediol, and is reduced still further

by the presence of the water (usually 3%) and other substances that are present in the commercial product. To prevent solidification in the container prior to use, water or other substances could be added to *levo*-2,3-butanediol, 15 to 20% water giving ample protection to temperatures of -20°C .

The freezing point curve for aqueous solutions of *meso-dextro*-2,3-butanediol (Fig. 2) is characterized by the formation of a *meso*-hydrate that crystallizes at temperatures well above the freezing point of water, approaching that of the anhydrous diol mixture. The presence of the *dextro*-form leads to a general reduction of the freezing points, but the various aspects of the freezing-point-diols-concentration data arise from the changing nature of the solid phase, which is successively water, *meso*-hydrate, and anhydrous diol. Beginning at 0% diol (Fig. 2) the freezing point is depressed by the addition of the

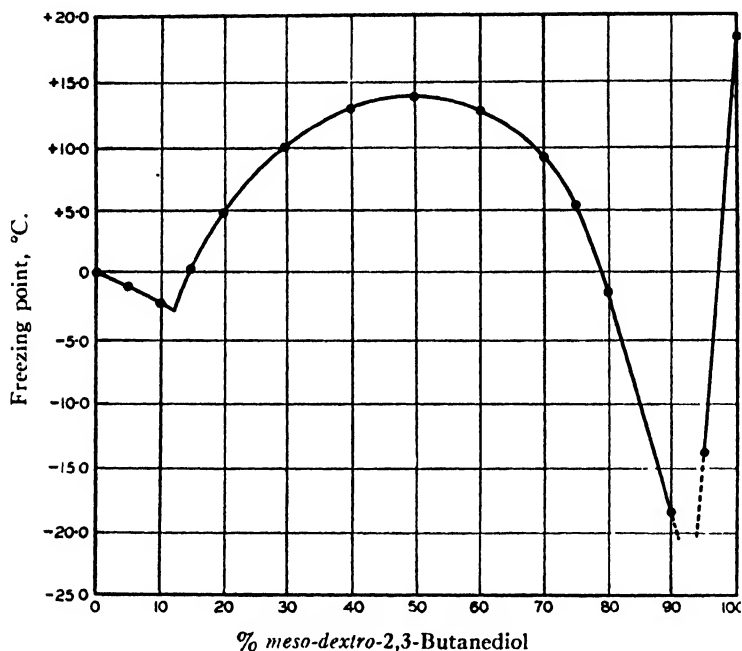


FIG. 2. Freezing points of aqueous *meso-dextro*-2,3-butanediol solutions.

diol mixture up to concentrations of 12%, the solid phase here being ice, there being sufficient water over this range of concentrations to keep the hydrate in solution at the equilibrium temperatures. The addition of further diol saturates the system with *meso*-hydrate which now becomes the solid phase, the freezing point of the mixture rising as the proportion of free or solvent water is reduced. The highest point on the freezing point curve represents maximum hydration, which is attained at 50% diol. With additional diol the freezing point of the *meso*-hydrate is depressed to a minimum, which is attained at 90% diol. Beyond this eutectic, the solid phase is

anhydrous diol, the freezing point rising sharply as the quantity of solvent hydrate is reduced. Sixty per cent aqueous solutions of the diol mixtures produced by *Aeromonas hydrophila* and *Bacillus subtilis* showed freezing points of +12.8° and +5.2° C., respectively, the freezing point value dropping as the proportion of *meso*-isomer is reduced. It should be noted that the present data are entirely at variance with the earlier statements made by Wilson and Lucas (36) on the freezing point characteristics of aqueous *meso*-2,3-butanediol solutions.

The high freezing temperature of aqueous *meso*-2,3-butanediol solutions has considerable bearing upon the practicability of employing the *levo*-isomer as an antifreeze. Contamination of *A. polymyxa* cultures with other butanediol-producing organisms could render the product useless as an antifreeze commodity. It is conceivable that races or variants of *A. polymyxa* exist that produce a mixture of isomers or could do so either by mutation or under special environmental conditions. In order to free the *levo*-form from esters, it is necessary to add alkalis before rectifying, with consequent possibilities of racemization. This contingency also exists in the automobile radiator, particularly under the mildly alkaline conditions that are often imposed to reduce the corrosion of metals by water. These potential sources of difficulty have been examined by the experiments that follow.

TABLE II

PHYSICAL PROPERTIES OF *levo*-2,3-BUTANEDIOL SAMPLES OBTAINED FROM DIFFERENT STRAINS OF *Aerobacillus polymyxa*

Organism	Alkali treatment*	Polarization value, 1 dm., at 25° C.	Freezing point, °C.	F.p. of 70% aqueous solution, °C.
C38 (2)	None	-12 60	+16 1	-42 8
C38 (2)	NaOH	-12 53	+16 1	-42 8
C42 (2)	None	-12 68	+16 1	-44.5
C8 (1)	Ca(OH) ₂	-12 33	+15 7	-42.5
C4, 21, 25 (mixed strains)	None	-12 55	+16 0	-41 3
C4, 21, 25	Ca(OH) ₂	-12 35	+15 5	-41.0
C4, 21, 25	NaOH	-12 45	+15.9	-42.2

* Fermented wheat mash adjusted to pH 10 0-10 5 before filtration and diol recovery.

The low optical rotation values of the diol samples referred to in Table II are attributed to the pronounced effect of small amounts of water on the optical rotatory power of this compound (5). Significant differences in freezing point lowering were not observed among butanediol samples obtained from different strains of *A. polymyxa*, regardless of whether they were or were not exposed to calcium hydroxide and sodium hydroxide in the recovery process (Table II). This observation has been borne out by subsequent experience, the products obtained with many different strains of *A. polymyxa* at the butanediol pilot plant having consistently shown the antifreeze characteristics

depicted in Fig. 1. While our experience does not entirely rule out the possible appearance of the *meso*-form in the *A. polymyxa* fermentation or in the subsequent stages of recovery, the chances for such an occurrence would now seem to be remote.

The influence of the *meso*-isomer on the freezing point of *levo*-2,3-butanediol solutions of uniform water content is illustrated by the experiment reported in Table III. At a water content of 40%, the freezing point is raised when the

TABLE III
EFFECT OF *meso*-2,3-BUTANEDIOL ON THE FREEZING POINT OF
AQUEOUS *levo*-2,3-BUTANEDIOL SOLUTIONS HAVING
WATER CONTENTS OF 40 AND 60%

Composition of diol	40% water	60% water
100% <i>levo</i>	-40.4° C.	-19.4° C.
95% <i>levo</i> 5% <i>meso</i>	-37.0	-21.0
90% <i>levo</i> 10% <i>meso</i>	-28.2	-21.0
85% <i>levo</i> 15% <i>meso</i>	-18.6	-17.2
80% <i>levo</i> 20% <i>meso</i>	-14.0	-12.4
50% <i>levo</i> 50% <i>meso</i>	+1.55	+1.55

meso-isomer is present to the extent of 5% of the total diol. The freezing point of solutions having water contents of 60% is actually lowered slightly by the presence of small amounts of the *meso*-form, but it rises quite sharply when this isomer makes up 15% or more of the total diol content.

The possibility of a *levo-meso* transformation in boiling aqueous solutions to which alkali had been added was examined by the experiment reported in Table IV. Adjustment of the pH of 50% aqueous solutions to values as high as 10.5 followed by 72 hours' refluxing did not result in the appearance of the *meso*-isomer, as judged by freezing point and optical rotatory power, both of these properties remaining substantially unchanged. The freezing point of

TABLE IV
OPTICAL ROTATORY POWER AND FREEZING POINT OF 50% *levo*-2,3-
BUTANEDIOL SOLUTIONS AFTER 72 HR. REFLUXING TREATMENT

Initial pH	Optical rotation, 2 dm.	F.p. °C.
6.95	-8.53	-34
8.90	-8.38	-33
10.82	-8.38	-33

levo-2,3-butanediol solutions was also determined after use in the cooling systems of a fleet of light trucks throughout the winter of 1944-45, the diol concentration being estimated by reference to refractive index tables (5). The observed freezing points in all cases agreed with the values shown in Fig. 1 within $\pm 0.5^\circ \text{C}$. The driving tests were entirely satisfactory in so far as operation of the motors was concerned.

Viscosity

The rate of circulation and general effectiveness of radiator solutions as coolants at very low temperatures is largely governed by their viscosity. Since the viscosity coefficient increases quite regularly with rising boiling point within series of homologous liquids, the advantage of permanence or high boiling point offered by aqueous solutions of glycerol or ethylene glycol might be expected to entail a considerable increase in viscosity over that of ethanol or methanol solutions. With the exception of the data provided by Cummings (7) for solutions of commercial glycerine, Prestone, and denatured alcohol at temperatures down to -8°C ., the available viscosity data for organic antifreeze fluids are limited to temperatures of 0°C . and upwards, most of the measurements referring to temperatures of 20°C . and higher. The changes in viscosity with concentration and temperature are very pronounced with both the non-volatile and volatile chemicals but there the resemblance stops. Addition of water to ethylene glycol or glycerol reduces the viscosity coefficient very drastically, evidently as a result of diminished association between like molecules. Addition of water to ethanol or methanol results in a very pronounced increase, evidently as a result of solvent-solute association. At 0°C . the viscosity of 40% ethanol is roughly four times that of either pure component and is approximately the same as that of 40% ethylene glycol at the same temperature, notwithstanding the fact that pure ethylene glycol is 17 times more viscous than ethanol at 20°C . (13). From the broad indications that are provided by existing data at high temperatures and experience gained in freezing point determinations, the order of increasing viscosity for liquid compositions freezing in the temperature range -30° to -50°C . is apparently methanol, ethanol, ethylene glycol, and glycerol, the viscosity of the glycerol solutions being much higher than that of the remaining three. Between ethylene glycol, methanol, and ethanol solutions the differences in viscosity are probably not large enough to be of practical importance.

The literature contains no information as to permissible viscosities for radiator solutions at low temperatures. In the absence of suitable standards, a viscosity of 500 centistokes has been selected as representing a level at which the initial circulation rate would not be retarded to an objectionable extent, and of 1000 centistokes as the upper limit beyond which circulation would be seriously impeded. From the flow properties observed in the course of freezing point determinations, Olsen *et al.* (25) concluded that 58% glycerol would circulate in the radiator system at -42°C . Our observations on the viscosity

of glycerol solutions (Fig. 4) indicate a viscosity value of around 1500 centistokes at this concentration and temperature, which is half as high again as the limiting value selected for our purposes.

The effect of water content on the absolute viscosity at 20° C. of glycerol, *meso-dextro*-2,3-butanediol, *levo*-2,3-butanediol, and ethylene glycol solutions is shown in Fig. 3. Past work on the viscosity of liquids (13) has established general relations between molecular structure and flow properties that have

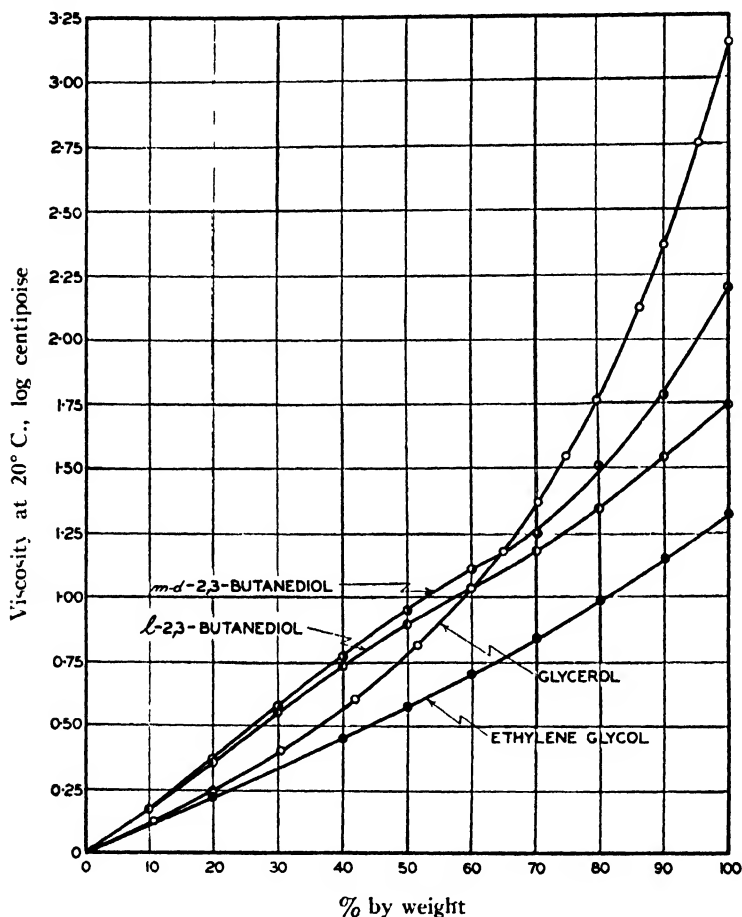


FIG. 3. Absolute viscosity of aqueous solutions of ethylene glycol, *levo*-2,3-butanediol, *meso-dextro*-2,3-butanediol, and glycerol at 20° C. Data for glycerol are taken from tables published by M. S. Sheely (29).

an immediate bearing on the very marked dissimilarities reported in this figure: hydroxyl groupings lead to association of like molecules in pure liquids and to hydrate formation in aqueous solutions; additional CH₃ groupings on otherwise similar molecules raise the viscosity coefficient by an amount that can be predicted by the use of equations; and differences in the viscosity of liquid stereoisomers are caused by differences in free space, the isomer

having the higher viscosity also having the higher density. Among the anhydrous liquids (Fig. 3), ethylene glycol has the lowest viscosity. This liquid is known to be highly associated in the absence of water (13). The 2,3-butanediols show higher values; this is to be expected in view of the two additional CH_3 groupings. The *meso*-form is more viscous and also has a higher density than the *levo*-form. This accords with the free space concept. The very high viscosity of anhydrous glycerol, i.e., 1499 centipoises at 20°C . (29), arises from its pronounced association and low free space. In an extensive study of the volume-temperature-pressure relations of liquid hydrocarbons, alcohols, glycols, and glycerol, Bridgman (3) found that, of all compounds tested, glycerol had the lowest compressibility. Some indication of the differences between pure *levo*-2,3-butanediol and glycerol in this respect is provided by their densities, which stand in the ratio of 0.99 to 1.26.

The sagged form of the viscosity-concentration curves for ethylene glycol and glycerol solutions (Fig. 3) indicates that dissociation resulting from admixture of highly associated liquids has a predominant influence on their fluidities, obscuring the effects of hydration, which occurs in both these systems. The outstanding feature of the curves for the 2,3-butanediols (Fig. 3) is their convexity at water contents of 20% and higher, the behaviour of both isomers being substantially similar. For this there appears to be but one possible explanation: within the indicated range of concentrations, the effects of dissociation are counterbalanced by hydration, the latter tending to increase the viscosity. Judging from the viscosity data, the *levo*- and *meso*-isomers are both characterized by a strong affinity for water; the remarkable differences in their antifreeze characteristics result from their differing solubilities in the hydrated state. Because of accompanying hydration, 2,3-butanediol solutions at concentrations of 50% or less are more viscous than glycerol solutions of corresponding concentration. Thus while hydration improves the antifreeze characteristics of the *levo*-isomer from the standpoint of freezing point lowering, the accompanying effect on viscosity largely offsets this advantage.

Fig. 4 presents comparative kinematic viscosity data for 60% solutions of *levo*-2,3-butanediol, glycerol, and ethylene glycol over the temperature range $+20^\circ\text{C}$. to -40°C . The butanediol solution is more viscous than that of glycerol at low temperatures, this difference becoming quite pronounced at -30°C . to -40°C . The viscosity of the ethylene glycol solution is much lower, the present data indicating that a value of 500 centistokes would be attained only at temperatures below -50°C . This viscosity value is shown by 60% glycerol at -33°C ., and by 60% *levo*-2,3-butanediol at -25.5°C . (Table V). A value of 1000 centistokes is attained by 60% glycerol at -39°C . and by 60% *levo*-2,3-butanediol at -30°C . When both freezing point lowering and viscosity are taken into consideration, 50% *levo*-2,3-butanediol appears most suitable for general use (Table V).

The decrease in viscosity that accompanies rising temperature is much more pronounced with organic antifreeze solutions than with pure water, the differences in fluidity between solution and pure solvent becoming negligible

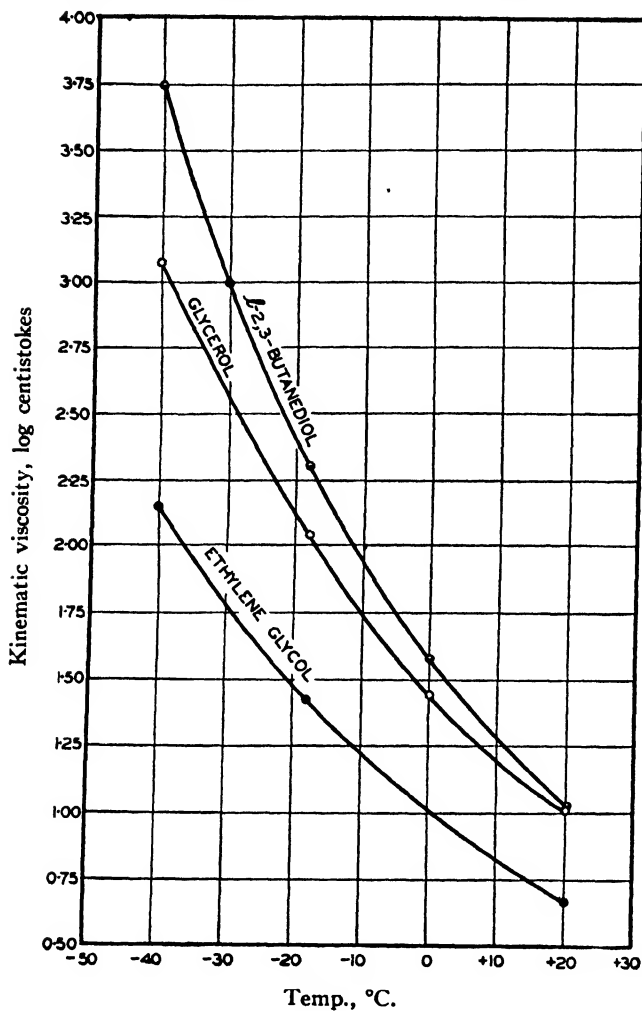


FIG. 4. Kinematic viscosity of 60% levo-2,3-butanediol, glycerol, and ethylene glycol solutions at low temperatures.

TABLE V

TEMPERATURES AT WHICH VISCOSITY VALUES OF 500 AND 1000 CENTISTOKES ARE ATTAINED BY AQUEOUS SOLUTIONS OF levo-2,3-BUTANEDIOL

levo-2,3- Butanediol, %	Temperature, °C.	
	500 centistokes	1000 centistokes
80	-18	-24
70	-22	-27
60	-26	-30
50	-29	-34

in so far as engine performance is concerned after a few minutes' operation of the motor. The converse is true of inorganic antifreeze solutions (13); with these the relative viscosity, referred to that of water at the same temperature, increases to a quite pronounced extent with rising temperature. Fig. 5 demonstrates the changes in viscosity of aqueous *levo*-2,3,-butanediol solutions

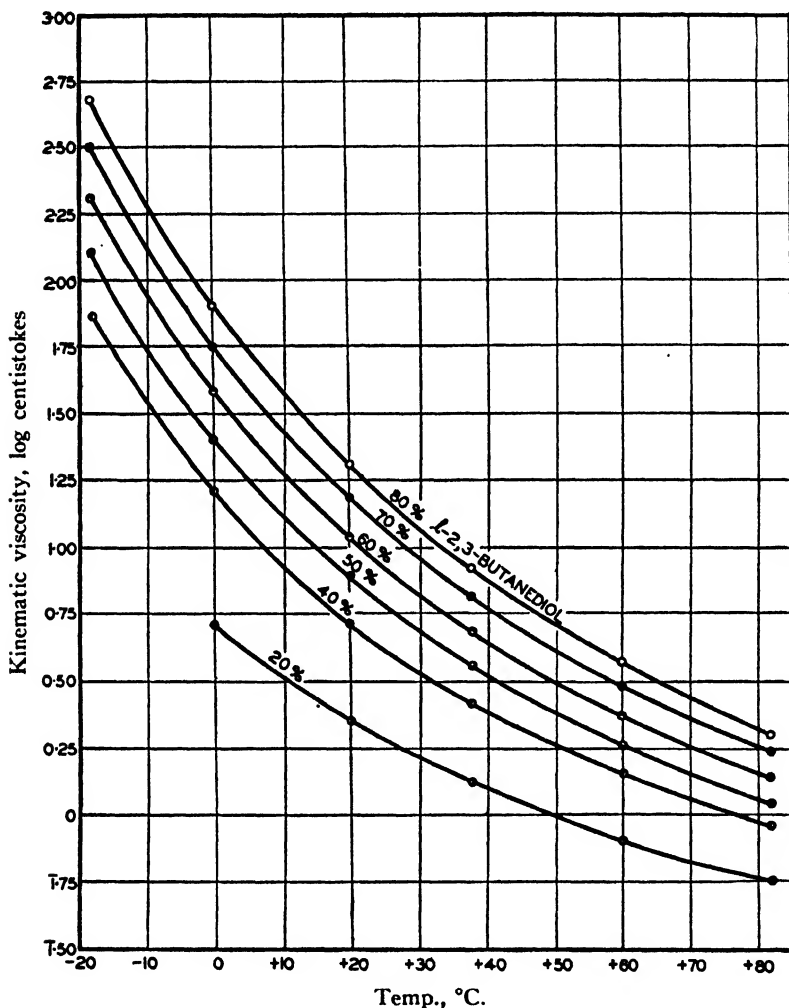


FIG. 5. Kinematic viscosity of aqueous *levo*-2,3-butanediol solutions, expressed logarithmically, as a function of concentration and temperature.

that occur when the temperature is increased from -20°C. to $+82^{\circ}\text{C.}$, this temperature range including the usual radiator temperatures during winter driving ($+60^{\circ}$ to $+71^{\circ}\text{C.}$) as well as the temperature normally attained immediately after the motor is shut off ($+82^{\circ}\text{C.}$). Fig. 6 illustrates the effects of diol concentration and temperature over a more limited range, the data

being presented in this figure as kinematic viscosity units for purposes of convenient reference.

Heat Capacity

Since automobile cooling systems are designed for water, high specific heats are desirable in antifreeze solutions. Information on the specific heat of 2,3-butanediols is at present limited to that provided by Khokhlovkin and

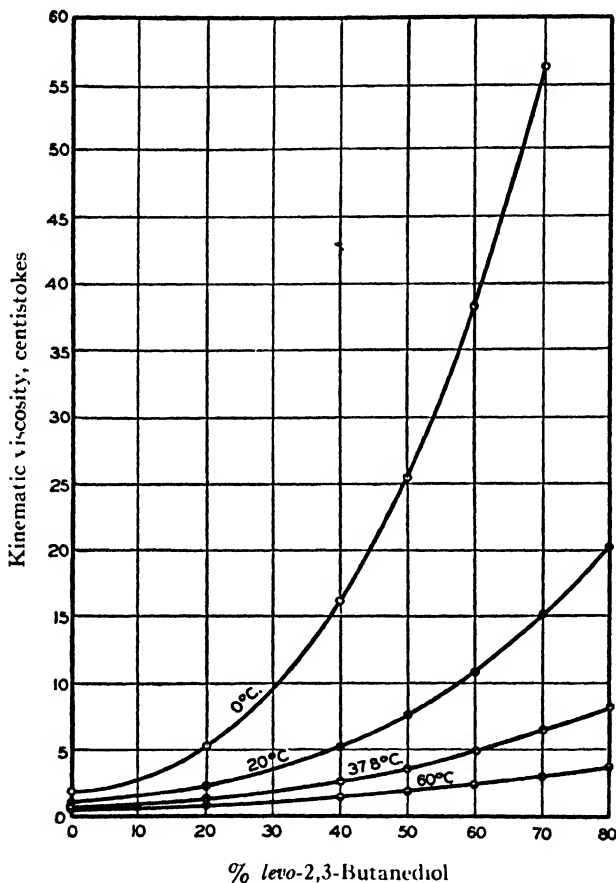


FIG. 6. Kinematic viscosity of aqueous levo-2,3-butanediol solutions in relation to concentration and temperature.

Kalacheva (19). The butanediol values given in Table VI were obtained from this source, that of the aqueous solution being only a rough estimate. Values for the remaining antifreeze chemicals in this table were calculated using data obtained from other sources (15, 24, 31).

From the comparative data presented in Table VI, it is apparent that butanediol does not differ greatly from ethylene glycol or glycerol with respect to specific heat, and that solutions of ethanol and methanol at concentrations

of 50% by weight have a specific heat much closer to that of water than similar concentrations of butanediol, ethylene glycol, and glycerol. Cummings (7) has pointed out that heat capacity per unit volume is more useful as an

TABLE VI
SPECIFIC HEATS OF ANTIFREEZE CHEMICALS AT 30° C.

	Anhydrous	50% by wt. aqueous solution
Methyl alcohol	0.61	0.91
Ethyl alcohol	0.59	0.93
Ethylene glycol	0.58	0.80
Glycerol	0.575	0.78
2,3-Butanediol	0.60	0.80

index of antifreeze cooling properties, and, on this basis, the discrepancy between the non-volatile and volatile chemicals would be considerably reduced. Judging from engine dynamometer tests reported by Green, Lamprey, and Sommer (12), engine performance actually is not altered significantly when solutions of ethylene glycol, ethanol or methanol are substituted for water as the cooling agent.

Surface Tension

Knowledge of the surface tension of antifreeze solutions provides information on their creeping and foaming tendencies. Liquids of low surface tension have a greater tendency to escape through small crevices in the cooling system than water, and as a general rule their solutions have a stronger foaming tendency (10). Untreated ethylene glycol solutions have already been shown by empirical test to leak more readily than water (12). The surface tension of pure *levo*-2,3-butanediol (Fig. 7) is considerably lower than that of ethylene glycol. This is to be expected from Traube's rule, the surface tension generally tending to drop off with increasing molecular weight among related chemicals. The shape of the butanediol graph conforms with that generally shown by aqueous solutions of capillary-active substances, a low concentration causing a pronounced lowering of the surface tension, intermediate and high concentrations reducing the value gradually to that of the pure liquid (10). From Gibbs rule, *levo*-2,3-butanediol should have a greater tendency to concentrate at the liquid-air interface than ethylene glycol.

Agents are customarily added to ethylene glycol solutions to reduce foaming and seepage during service (12). The present data indicate that similar precautions may be desirable when *levo*-2,3-butanediol is employed, although it is yet too early to assess the practical importance of the differences reported in the accompanying figure.

Corrosion

Methanol and glycerol were at first marketed for use in automobile radiators in very impure form, the free acids and electrolytes that they contained serving

to promote corrosion. This fault was soon corrected but the popular impression has persisted that antifreeze chemicals stimulate corrosion and that corrosion inhibitors are necessary adjuncts. A host of corrosion inhibitors

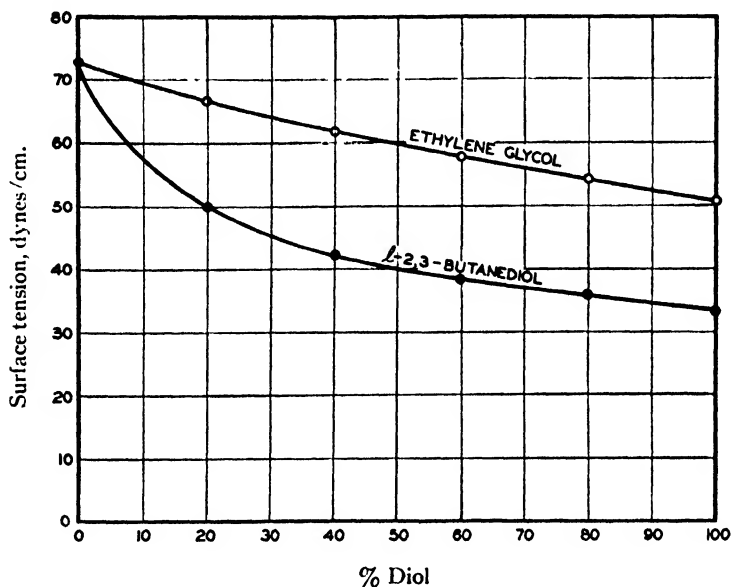


FIG. 7. Surface tension of aqueous solutions of *levo*-2,3-butanediol and ethylene glycol at 25° C.

has been patented for use in organic antifreeze solutions (33), but published information on this subject (7, 8, 18, 26, 37) shows agreement in indicating that water alone is at least as corrosive as aqueous solutions of the common organic antifreeze chemicals.

TABLE VII

CORROSION OF STEEL PANELS BY TWO MONTHS' TOTAL IMMERSION IN AQUEOUS ANTIFREEZE SOLUTIONS

Sample	Weight loss, gm./sq. in.
50% <i>levo</i> -2,3-butanediol	0.0122
50% <i>levo</i> -2,3-butanediol containing 0.1% NaNO ₂	None
50% ethylene glycol	0.0190
50% "Prestone"	0.0002

Table VII reports a comparison of the corrosive action on steel of aqueous solutions of *levo*-2,3-butanediol and ethylene glycol with and without nitrite inhibitor. In the absence of nitrite, the steel panels were attacked by both solutions to an approximately equivalent extent. In the presence of 0.1% sodium nitrite the corrosion of steel by aqueous *levo*-2,3-butanediol was

eliminated, as was also the case with commercial ethylene glycol solution containing the inhibitor or inhibitors customarily added.

Table VIII reports an experiment in which test plates of steel, brass, aluminum, and lead were bolted together and then immersed for two months in 50% *levo*-2,3-butanediol solution containing 0.1% sodium nitrite. The attack

TABLE VIII

CORROSION OF STEEL, BRASS, ALUMINUM, AND LEAD BY 50% *levo*-2,3-BUTANEDIOL CONTAINING 0.1% SODIUM NITRITE (PANELS BOLTED TOGETHER DURING TWO MONTHS' IMMERSION)

Panel	Weight before test, gm.	Weight after test, gm.	Weight change	Weight change, gm./sq. in.
Steel	13.3072	13.3066	-0.0006	-0.0001
Brass	15.7191	15.7235	+0.0044	+0.0009
Aluminum	4.9495	4.9528	+0.0033	+0.0007
Lead	27.7316	27.1652	-0.5664	-0.1135

on steel, brass, and aluminum is judged of no practical importance. Lead was very strongly attacked, and subsequent tests showed that this arose from the use of distilled water in making up the solutions. Ethylene glycol solutions made up in the same way cause quite as much damage. The corrosive action of pure water on lead has been satisfactorily explained by Thresh (32, 33) and is also discussed by Evans (9, p. 98): in the absence of sulphates or bicarbonates the lead passes freely into solution, whereas in ordinary tap water an impervious protective film of lead sulphate or carbonate is formed over the exposed surface. Cummings (7) has suggested the use of soft water in radiators as a means of reducing scale formation. It should be noted that this practice might lead to a greater corrosion of soldered joints than occurs when tap water is employed.

If special precautions are not taken, *levo*-2,3-butanediol is contaminated with esterified acetic acid in the course of its recovery from fermented mashes. In the foregoing corrosion experiments, the ester content of the diol was 1.0%, expressed as monoacetate, and from the present results this concentration appears to be permissible. Since the acetate is partially hydrolysed in the presence of water, the ester content should be kept as low as possible in butanediol intended for use as an antifreeze.

Solvent Action on Automobile Finishes

Ethanol and methanol have a strongly deteriorative effect on the protective coatings of the engine hood (7), but this property has been claimed to be unimportant when the alcohols are considerably diluted with water (8). Since objection to ethylene glycol has never been taken in this respect, its deteriorative action on typical automobile finishes (nitrocellulose lacquer, "Dulux", and alkyd base enamels) has been compared with that of *levo*-2,3-butanediol (Table IX). All three finishes were softened to some extent by

TABLE IX

SOLVENT ACTION OF *levo*-2,3-BUTANEDIOL AND ETHYLENE GLYCOL ON AUTOMOBILE FINISHES

Metal finish	<i>levo</i> -2,3-Butanediol		Ethylene glycol	
	60%	100%	60%	100%
Nitrocellulose lacquer	2	3	2	2
"Dulux" enamel C.I.L.	3	2	3	2
Alkyd base enamel	4	4	3	3

Rating of the physical condition of metal finishes after exposure: 1—good, 2—fair, 3—poor, 4—bad.

exposure to these antifreeze compounds, water content having little effect over the tested range of concentrations. Ethylene glycol tended to be slightly less deteriorative than *levo*-2,3-butanediol, but the observed differences in this respect are not judged large enough to have practical significance. The results indicate (Table IX) that precautions should always be taken to prevent exposure of automobile finishes to antifreeze compounds of this type, and that alkyd base enamels are more easily damaged in this way than nitrocellulose lacquers.

Effects on Natural and Synthetic Rubber

One of the most objectionable characteristics of oils and kerosene as engine coolants is their strongly deteriorative action on rubber hose connections (7, 18, 37), the same being generally true of oil-type corrosion inhibitors (12). Technical data are lacking on the relative suitability of aqueous antifreeze solutions in this respect. Green *et al.* (12) conclude that hose failures are primarily related to the grade of rubber and type of hose employed, rather than to the type of aqueous antifreeze solution that is used in the cooling system. They report that radiator hose connections remain serviceable for a longer time with commercial ethylene glycol solutions than with water. This is somewhat at variance with the statement of Keyes (18), who cited rubber-softening as a disadvantageous characteristic of ethylene glycol.

Table X reports the changes in various properties of natural and synthetic rubber that resulted from three days' immersion in 60% *levo*-2,3-butanediol and ethylene glycol solutions at 80° C. The change in tensile strength was approximately the same with both antifreeze solutions, Buna S being affected to a greater extent. The "elongation at break" test on both rubber compounds indicated a slight superiority for the butanediol solution. Neither solution reduced the hardness of Buna S appreciably, and the hardness of natural rubber was affected only to a small extent by the butanediol solution. The butanediol solution had a considerably greater swelling action on both types of rubber. According to T. R. Griffith of the National Research Council rubber laboratory, a 20% volume increase would not materially affect the serviceability of rubber hose connections, and in his opinion the observed

TABLE X

EFFECT OF 60% ETHYLENE GLYCOL AND 60% *levo*-2,3-BUTANEDIOL ON NATURAL AND BUNA S RUBBER COMPOUNDS (THREE DAYS' IMMERSION AT 80° C.)

	Tensile strength (lb./sq. in.)	Elongation at break, %	Volume change, %	Hardness number
<i>Natural rubber</i>				
Untreated	3075	645	0	51
Ethylene glycol	2800	595	+ 1.5	49
<i>levo</i> -2,3-Butanediol	2820	635	+11.9	46
<i>Buna S rubber</i>				
Untreated	2850	615	0	56
Ethylene glycol	2000	375	+ 5.9	54
<i>levo</i> -2,3-Butanediol	2070	410	+10.7	54

differences in swelling action do not detract from the usefulness of *levo*-2,3-butanediol as an antifreeze compound.

Permanence

Fig. 8 demonstrates that the boiling points of aqueous *levo*-2,3-butanediol solutions are essentially the same as those of ethylene glycol and glycerol of equivalent concentrations. Barring losses by leakage and by overflow after

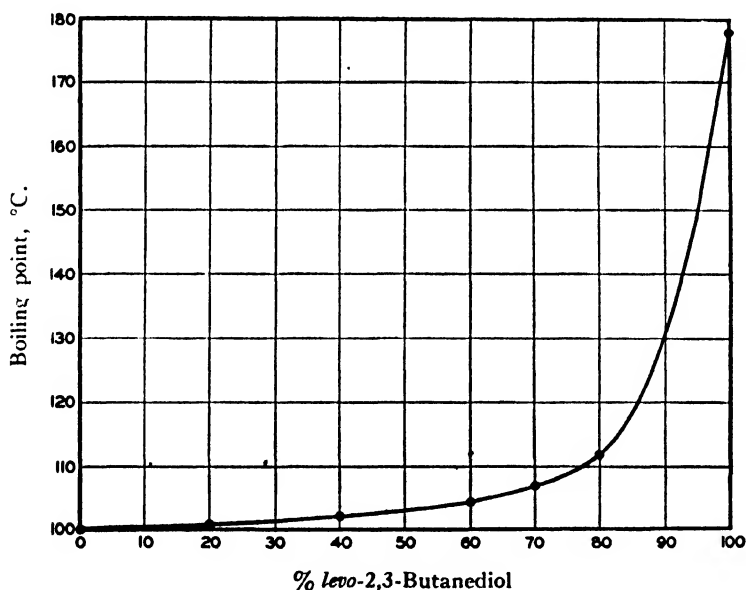


FIG. 8. Boiling points of aqueous *levo*-2,3-butanediol solutions at atmospheric pressure.

the motor is shut off, only water need be added to maintain a uniform diol concentration. As with other permanent-type antifreeze compounds, the solution could be saved for re-use.

Inflammability

The inflammability of ethanol and methanol imposes a fire hazard, the open-cup flash points of the pure chemicals being below room temperature (15° to 16° C.), while that of fairly concentrated aqueous solutions is well below usual radiator temperatures. The open-cup flash point of *levo*-2,3-butanediol is 110° C., which is similar to the value that has been reported elsewhere (2) for ethylene glycol (116° C.). With either of the last two substances the presence of small amounts of water would increase the flash point very considerably.

Solubility

The solubility-temperature relations for aqueous *levo*-2,3-butanediol solutions containing various third components will be treated in detail in a forthcoming publication (6). The present chemical is miscible in all proportions with any of the common antifreeze chemicals, their antifreeze properties in admixture being approximately additive.

Expansion on Solidification

The freezing point of organic antifreeze solutions is the highest temperature at which crystals can be induced to separate on seeding. As the temperature is lowered further, the liquid becomes "mushy", but the small accompanying expansion due to ice formation is of no practical consequence since the system is still semifluid. The importance of expansion when the solution becomes solid has been tested by filling Erlenmeyer-shaped weighing bottles, having thin glass walls, with 40% *levo*-2,3-butanediol and ethylene glycol solutions and solidifying the contents at temperatures 20° C. below their freezing points. The fact that the glass vessels did not rupture demonstrated that solidification of these solutions in the radiator would not lead to damage by expansion.

Thermal Expansion

A low coefficient of thermal expansion is desirable in antifreeze fluids (18, 37), and in this respect *levo*-2,3-butanediol and its solutions compare favourably with ethylene glycol. Using the simplified expression $V_t = V_0(1 + \alpha t)$, comparable values for $\alpha \times 10^3$ over the temperature range 20° to 40° C. are: water, 0.3; 60% *levo*-2,3-butanediol, 0.79; *levo*-2,3-butanediol, 0.85; 60% ethylene glycol, 1.15; anhydrous ethylene glycol, 0.90; ethyl alcohol, 1.15. The specific gravity data on which the *levo*-2,3-butanediol figures are based are presented elsewhere (5).

Density

The density of antifreeze solutions is of interest for two reasons: with comparatively heavy liquids such as glycerol, the antifreeze chemical sinks to the bottom of the radiator if it is not mixed with water beforehand; because of

the ease with which it may be measured, this property also forms the basis of practical tests for freezing point protection in used radiator solutions, ethylene glycol and glycerol solutions being much heavier, and alcohol solutions much lighter than water. From a practical standpoint it is impossible to determine the strength of *levo*-2,3-butanediol solutions by density measurements since the values for its solutions are very close to that of water at all concentrations.

Conclusions

levo-2,3-Butanediol and ethylene glycol are judged equally satisfactory as antifreeze compounds from the standpoints of stability, permanence, heat capacity, inflammability, thermal expansion, expansion on solidification, and deteriorative action on metals, metal finishes, and rubber hose connections. Admittedly, there are some differences in these respects, but on the whole they are judged too small to be of practical importance in the present application. Ethylene glycol solutions have higher surface tensions and lower freezing points than the corresponding *levo*-2,3-butanediol solutions. The differences observed in these two properties are considered to be of secondary importance in view of the relative ease with which creeping and air-entrainment tendencies may be controlled and the fact that temperatures below -40°C . are seldom encountered.

The detrimental properties of *levo*-2,3-butanediol solutions are high viscosity at low temperature and uniform density at all concentrations. It is considered unlikely that difficulties would arise from high viscosity except at temperatures below -30°C . It should be noted that *levo*-2,3-butanediol solutions are rendered considerably less viscous by the addition of third components, and may then be used at a temperature of -50°C . (6). The problem of testing butanediol solutions after use for diol concentration has been satisfactorily solved by co-workers who have devised and calibrated a practical instrument suitable for use in service stations.

The most suitable concentration for use in automobile radiators is judged to be 50%, and this strength has proved satisfactory in large scale driving tests. It may prove advantageous to add anticreep and antifoam agents although no need for them has been indicated so far in driving tests. Corrosion inhibitors such as sodium nitrite might also be added if there is greater need for rust inhibitors during the winter months than during the remaining seasons when water alone is used.

The future of *levo*-2,3-butanediol as an antifreeze rests with production costs and concurrent prices of competitive chemicals. According to Callahan (4), the *meso*-isomer made by fermentation of corn or wheat with *Aerobacter aerogenes* could probably compete successfully as a source of butadiene with ethyl alcohol obtained from the same raw materials: the yields of alcohol and of the *meso*-isomer are of the same order, and the higher yields of butadiene that are obtained with the diol would largely compensate for the higher cost of recovering the diol from fermented mash. Existing information on the production of *levo*-2,3-butanediol with *Aerobacillus polymyxa* is largely restricted

to the use of buffered grain mash as substrate, the yield of diol in this instance being lower than in the *Aerobacter aerogenes* fermentation. Although the *levo*-isomer could not be made as cheaply as ethyl alcohol or the *meso*-form, it could probably be marketed at a retail price considerably below the average price of ethylene glycol over the past 10 years. If the price of ethylene glycol was reduced greatly, *levo*-2,3-butanediol could remain in a competitive position only through technological improvements and adaptation of the process to cheaper raw materials. The antifreeze trade offers a considerable outlet for chemicals derived from plant products, but its limitations must be recognized. Judging from the volume of pre-war trade in antifreeze chemicals, the entire Canadian demand could be satisfied by a butanediol industry having an annual capacity of one, or at most two, million bushels of wheat.

Acknowledgments

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THE PROBLEM OF HEAT ADDITION IN DUCTS¹

By D. G. SAMARAS²

Abstract

The one-dimensional problem of heat addition in a duct with friction losses is examined.

The variation of the total head temperature and pressure ratio, as well as the static ones, have been obtained as functions of the Mach number and the frictional coefficient. It is shown that an extreme value of the total head temperature and pressure ratio is obtained when the Mach number equals unity. An extreme value of the static temperature is obtained at Mach numbers smaller or larger than unity, depending on the form of the duct.

It is concluded that when the total head pressure losses are to be kept low, a diverging duct must be used. The frictional losses have a convergence effect on the flow. When the friction losses are disregarded, no total head pressure loss will result if the area variation of the duct is such as to ensure a constant Mach number.

The results and conclusions found may be useful in the design of combustion chambers for gas turbines, propulsive ducts, and rockets.

Notation

The following symbols have been used in the analysis:

A	=	Cross-sectional area,
C_f	=	Friction coefficient,
g	=	Gravitational constant,
K	=	Constant,
\ln	=	Naperian logarithm,
M	=	Mach number,
m	=	Exponent of the cross-sectional area variation,
p	=	Absolute pressure,
Q	=	Air mass-flow,
R	=	Radius of the cylindrical duct,
r	=	Radius of the cross-section (general case),
R_c	=	Gas constant,
Rel	=	Refers to relative values of the appropriate quantities, usually to values at the inlet of the duct,
T	=	Absolute temperature,
V	=	Velocity,
x	=	Axial co-ordinate,
y	=	Temperature ratio,
Z	=	M^2 = square of the Mach number,
α	=	Auxiliary function of the cross-sectional variation and friction, used for convenience only,
γ	=	Isentropic exponent,

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- θ = Slope of the meridian of the duct wall,
 ρ = Density,
 τ = Shear stress (friction),
 ω = Auxiliary function of the rate of heat addition and friction in the cylindrical duct.

The following subscripts have been employed:

- $1,2$ = Inlet and outlet of the duct respectively,
 ex = Extreme value,
 s = For static,
 t = For total head.

1.0 Introduction

The maximum attainable temperature by heat addition in a duct has often been questioned. The simplified problem of a cylindrical duct with no losses was examined, and several conclusions in divergence with present day experience were drawn. That led the author, two years ago, to attempt a general solution of the problem. It is well known that heat addition in a gas stream results in an increase of the static temperature and in an increase of the velocity, both changes being reflected in the variation of the total head temperature.

From the above it is reasonable to deduce that, in a gas-stream, heat can be added without necessarily increasing the static temperature; also a maximum static temperature can be reached and still further heat can be added with decreasing static temperature, by accelerating the fluid, until the gas velocity at a certain section reaches the local velocity of sound, and "choking" is said to occur.

According to the above and in order to avoid the misleading influence of the static temperature, the total head temperature has been employed, since it gives a true picture of the process of heat addition; also for comparative reasons the static temperature variation has been included.

The general problem of designing a duct of given inlet and outlet temperatures and loss distribution is a very complicated one; it is a three-dimensional problem and it is reduced to a two-dimensional one in the case of axial symmetry. Still, the two-dimensional problem is so complicated that no attempt was made to attack it, as the general conclusions drawn from the one-dimensional problem can be applied to that with slight modifications.

In the present stage of development very little is known of the reaction mechanism of combustion of technological fuels; therefore a theoretical calculation of the combustion process is more or less impossible. Even in the case of simple fuels such as methane, the process of combustion is a very complicated one. It was found that during the combustion of methane several intermediate products are formed, such as methyl alcohol and formaldehyde; also $CC-$ and $CH-$ bands have been spectroscopically observed.

For many fuels the reaction process is fundamentally different at different temperatures; at low temperatures cool flames may appear that are entirely

absent at higher temperatures. To this may be added the possibility of a time dependence of the chain reactions. From the above-mentioned multiplicity of the combustion phenomena, two idealized cases of the reaction process can be distinguished; that is, the thermal reaction and the chain reaction. In both cases a large increase of the reaction velocity appears with the time; in the first by the increase of the temperature due to the liberated heat, and in the second owing to chain branching. In practice we have a combination of both.

In spite of the complicated combustion phenomena, some empirical formulae can be established that may render immense help to the designer, by simplifying the treatment of the combustion process. In a homogeneous gas mixture the mass rate of combustion is proportional to the concentrations of the gases taking part in the reaction and to a reaction velocity given by the well known Arrhenius formula. This formula can also be deduced from statistical considerations by using Boltzmann's distribution law.

Not only physico-chemical but also aerodynamical phenomena are present in the combustion in a duct and these make the problem even more complicated.

It was found that the flame velocity of a Bunsen burner in the laminar region is considerably lower than in the turbulent region. Now considering that in a duct a great amount of turbulence is to be expected, the results on the flame propagation are obvious.

In a duct, two types of turbulence are usually present; the small and large scale turbulence. Large scale turbulence in a duct is usually produced by the wakes of bodies, especially in the case of the Kármán vortex trails. Small scale turbulence is produced in a combustion chamber of a gas turbine engine by the compressor wakes and in a propulsive duct by the entry oblique shock-waves.

It can be concluded that the rate of heat addition in an actual duct as a function of the geometrical co-ordinates is unknown, and the most simplified assumptions of flame propagation and ignition lag would not contribute very much to the conclusions drawn. Therefore the geometrical co-ordinates were disregarded and instead the mean Mach number across the section has been employed.

Finally, the best geometrical form of a duct may be found by combining the conclusions of the analysis with experimental evidence.

2.0 Assumptions

As was mentioned above, necessary simplifying assumptions were made in order to evolve the general equations in as simple form as possible.

Although the velocity, temperature, and pressure variation at any section is considerable and variable with time, their mean values have been used in the analysis.

The increase of the mass flow due to fuel addition has been neglected. A mean specific heat, constant for all cases, has been assumed. A mean shear stress, corresponding to the total frictional loss, has been assumed as applied parallel to the wall of the duct. In reality, owing to turbulence the losses are distributed throughout the volume of the duct; but a spatial distribution would only complicate matters.

A circular duct has been assumed; but as the form of the cross-section enters only in the frictional term, by taking into consideration the former assumption the results can be applied to any form of cross-section where the friction losses are small.

It was found in the analysis that the cross-sectional area that corresponds to a constant Mach number is an exponential function of the total head temperature ratio. This led us to the very useful assumption, that the cross-sectional area is an exponential function of the total head temperature ratio

3.1.0 General Equations of Motion

From Fig. 1 it can easily be seen that the equation of momentum in the axial direction is as follows:

$$d \left\{ (p_s + \rho_s V^2) A \right\} - p_s dA + \tau \cdot 2\pi r dx = 0 \quad (1)$$

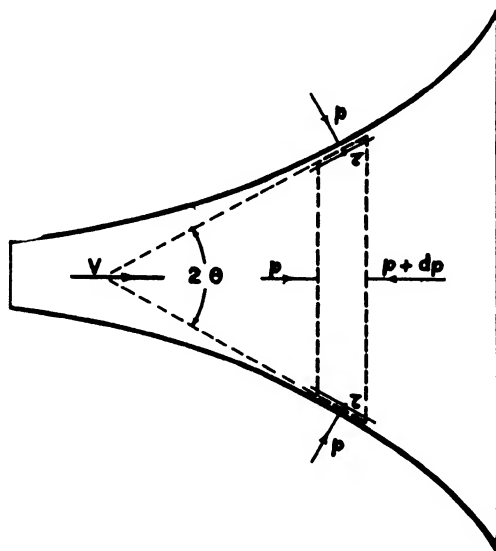


FIG. 1. Diagram of a duct.

The shear stress (friction) can be put in the form:

$$\tau = \frac{1}{2} C_f \rho_s \left(\frac{V}{\cos \theta} \right)^2. \quad (2)$$

The equation of continuity may be written as follows:

$$Q = g \rho_s V A. \quad (3)$$

The equation of state for perfect gases may be written in the form:

$$p_t = g\rho_t R_c T_t. \quad (4)$$

The section area variation has been assumed as:

$$A = \pi r^2 = Ky_t^m. \quad (5)$$

Now calling

$$Z = M^2, \frac{T_t}{T_{t1}} = y_t, \text{ then } V^2 = g\gamma R_c \frac{Z \cdot T_t}{1 + \frac{\gamma-1}{2}Z}.$$

3.2.0 The Equation of the Total Head Temperature Ratio

From the above equations the following can be obtained:

$$d \left\{ \rho_t V^2 A \left(1 + \frac{1}{\gamma Z} \right) \right\} - p_t dA + \frac{1}{2} C_f \rho_t \left(\frac{V}{\cos \theta} \right)^2 2\pi r dx = 0, \quad (6)$$

and finally, after some simple transformations as shown in Appendix I, we obtain

$$\frac{1-Z}{Z \left(1 + \frac{\gamma-1}{2}Z \right)} \cdot \frac{dZ}{dy_t} + 2 \left(1 - \frac{\gamma C_f}{\sin 2\theta} Z \right) \frac{d \ln A}{dy_t} = \frac{1 + \gamma Z}{y_t}. \quad (7)$$

3.2.1 The Ideal Case with No Friction Losses

In this case the friction coefficient $C_f = 0$, and Equation (7) becomes:

$$\frac{(1-Z)}{Z \left(1 + \frac{\gamma-1}{2}Z \right)} \cdot \frac{dZ}{dy_t} + 2 \frac{d \ln A}{dy_t} = \frac{1 + \gamma Z}{y_t}. \quad (8)$$

and the constant Mach number area variation is given by:

$$Rel(A) = [y_t]^{\frac{1+\gamma Z}{2}}, \quad (9)$$

Equation (8) in conjunction with Equation (5) gives:

$$\frac{(1-Z)}{Z \left(1 + \frac{\gamma-1}{2}Z \right)} dZ = [(1-2m) + \gamma Z] \cdot \frac{dy_t}{y_t}. \quad (10)$$

$$\text{By putting } \alpha_0 = \frac{\gamma}{1-2m} \quad (11)$$

and integrating Equation (10) we finally obtain

$$Rel \left\{ \frac{Z}{1 + \frac{\gamma-1}{2}Z} \right\} \cdot Rel \left\{ \frac{1 + \alpha_0 Z}{1 + \frac{\gamma-1}{2}Z} \right\}^{\left(\frac{1+\alpha_0}{\frac{\gamma-1}{2} - \alpha_0} \right)} = y_t^{(1-2m)}. \quad (12)$$

In the case of a cylindrical duct $m = 0$ and Equation (12) becomes:

$$Rel \frac{Z \left(1 + \frac{\gamma-1}{2}Z \right)}{(1 + \gamma Z)^2} = y_t. \quad (13)$$

3.2.2 The General Case

In the general case the same formulae are valid, with the difference that we use α instead of α_0 .

$$\alpha = \frac{\gamma}{1 - 2m} \left(1 + \frac{2m \cdot C_f}{\sin 2\theta} \right). \quad (14)$$

In the case of a cylindrical duct $m = 0$, and Equation (14) becomes indefinite, by modifying it as follows:

$$\frac{2m}{\sin 2\theta} = \frac{1}{\cos^2 \theta} \cdot \frac{2y_t}{r} \cdot \frac{dx}{dy_t} \quad (15)$$

we obtain:

$$\alpha^* = \gamma \left(1 + \frac{2y_t}{R} \cdot \frac{dx}{dy_t} \cdot C_f \right). \quad (16)$$

Calling

$$\omega = \frac{2\gamma}{\gamma + 1} \cdot \frac{y_t}{R} \cdot \frac{dx}{dy_t} \cdot C_f \quad (17)$$

we transform Equation (12) as follows:

$$y_t = \text{Rel} \left[\frac{Z \left(1 + \frac{\gamma - 1}{2} Z \right)}{(1 + \gamma Z)^2} \right] \cdot \text{Rel} \left[\frac{1 + \frac{\gamma - 1}{2} Z}{1 + [\gamma + (\gamma + 1)\omega] Z} \right]^{\frac{(2 + 2\omega)}{(1 + 2\omega)}} \cdot \text{Rel} \left[\frac{-(1 + \gamma Z)}{1 + \frac{\gamma - 1}{2} Z} \right]^2. \quad (18)$$

If moderate losses are assumed then $\omega \ll 1$, and Equation (18) is transformed into:

$$\text{Rel} \left[\frac{Z \left(1 + \frac{\gamma - 1}{2} Z \right)}{(1 + \gamma Z)^2} \right] = y_t \cdot \left\{ 1 + 2(\gamma + 1) \left[\frac{Z_2}{1 + \gamma Z_2} - \frac{Z_1}{1 + \gamma Z_1} \right] \right\}. \quad (19)$$

From Equations (13) and (19) it can be ascertained that the friction losses are equivalent to a convergence effect on the flow. We see from Equation (10) that an extreme value of y_t is obtained when $M = 1$, i.e., choking occurs.

3.3.0 The Total Head Pressure Ratio

From Equations (1) and (2) we obtain the following:

$$d \left\{ p_t \frac{1 + \gamma Z}{\left[1 + \frac{\gamma - 1}{2} Z \right]^{\frac{\gamma}{\gamma - 1}}} A \right\} - \left\{ \frac{p_t}{\left[1 + \frac{\gamma - 1}{2} Z \right]^{\frac{\gamma}{\gamma - 1}}} - \frac{C_f}{\sin 2\theta} \cdot \frac{\gamma p_t Z}{\left[1 + \frac{\gamma - 1}{2} Z \right]^{\frac{\gamma}{\gamma - 1}}} \right\} dA = 0, \quad (20)$$

and finally after some simple calculations as shown in Appendix II we obtain:

$$Rel\ p_t = Rel\ \left\{ \frac{\left[1 + \frac{\gamma - 1}{2} Z\right]^{\alpha(\frac{\gamma+1}{\gamma-1})}}{[(1 + \alpha Z)]^{(1+\alpha)}} \right\}^{\frac{1}{2\alpha+1-\gamma}} \quad (21)$$

From Equation (21) it is seen that if α is constant no pressure loss will occur if the Mach number is kept constant along the duct. From Equations (5) and (7) this will correspond to:

$$m = \frac{1 + \gamma Z}{2\left(1 - \frac{\gamma C_f Z}{\sin 2\theta}\right)} \quad (22)$$

where a mean value of $\frac{C_f}{\sin 2\theta}$ is to be taken. Substituting this in Equation (14) we obtain the following:

$$\alpha' = -\frac{1}{Z}. \quad (23)$$

3.4.0 The Static Temperature Ratio

The static temperature is found to be:

$$y_s = \frac{y_t}{Rel\left(1 + \frac{\gamma - 1}{2} Z\right)}, \quad (24)$$

and using Equation (12) we obtain:

$$y_s^{(1-2m)} = \frac{Rel\left[1 + \frac{\gamma - 1}{2} Z\right]}{Rel\left[1 + \frac{\gamma - 1}{2} Z\right]^{(1-2m)} \cdot Rel\left[1 + \frac{\gamma - 1}{2} Z\right]^{\left(\frac{1+\alpha}{\alpha - \frac{\gamma-1}{2}}\right)}}. \quad (25)$$

For an extreme value of y_s we differentiate and finally obtain:

$$Z_{ex}^2 \alpha(1 - 2m) - 2Z_{ex} \left[m - \frac{\gamma + 1}{2(\gamma - 1)}\right] - \frac{2}{\gamma - 1} = 0. \quad (26)$$

Solving Equation (26) we obtain

$$Z_{ex} = \frac{\left[m - \frac{\gamma + 1}{2(\gamma - 1)}\right] \pm \sqrt{\left[m - \frac{\gamma + 1}{2(\gamma - 1)}\right]^2 + \frac{2\alpha(1 - 2m)}{\gamma - 1}}}{\alpha(1 - 2m)}. \quad (27)$$

Only the positive root has a physical meaning.

3.5.0 The Static Pressure Ratio

The static pressure ratio is given by

$$Rel\ p_s = \frac{Rel\ p_t}{Rel\left[1 + \frac{\gamma - 1}{2} Z\right]^{\left(\frac{\gamma}{\gamma-1}\right)}} \quad (28)$$

and using Equation (21) we obtain:

$$Rel p_s = Rel \frac{\left[1 + \frac{\gamma-1}{2} Z\right]^{\left(\frac{\gamma-\alpha}{2\left(\alpha-\frac{\gamma-1}{2}\right)}\right)}}{\left[(1+\alpha Z)\right]^{\left(\frac{(1+\alpha)}{2\left(\alpha-\frac{\gamma-1}{2}\right)}\right)}}. \quad (29)$$

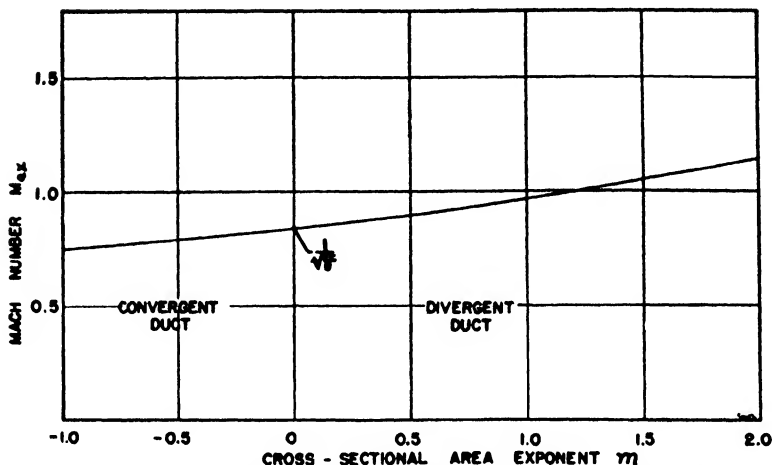


FIG. 2. Variation of the extreme Mach number with the cross-sectional exponent.

4.0 Results and Discussion

Fig. 2 shows the different values of the Mach number at which the static temperature reaches an extreme value, as a function of the exponent m associated with the cross-section. This holds in the absence of friction losses; when there are friction losses the small divergence effect of friction may be easily accounted for.

From Fig. 2 it is seen that M_{ox} increases continuously with increasing m . Thus for a cylindrical duct $m = 0$, $M_{ox} = \frac{1}{\sqrt{\gamma}}$, and for a divergent one for which $m = 1.2$, $M_{ox} = 1.0$.

Figs. 3, 5, 7, and 9 show the variation of the total head and static temperature ratios as a function of the Mach number for given exponents of the cross-section $m = 0, 0.5, 1.0$, and 1.5 respectively.

Figs. 4, 6, 8, and 10 show the variation of the total head and static pressure ratios as a function of the Mach number and the same exponents m .

From an inspection of the above curves, it can be seen that in a duct considerable amounts of heat may be added with small total head pressure loss if the duct is divergent enough and no increase in Mach number occurs.

In the subsonic region if the divergence is small (m small) addition of heat in the stream is followed by a simultaneous acceleration of the fluid and increase of the Mach number; on the other hand, if the divergence is large, heat may be

In the supersonic region, addition of heat in the stream is followed by a decrease of the Mach number, except when the relative divergence of the duct is very large and the initial Mach number approaches unity.

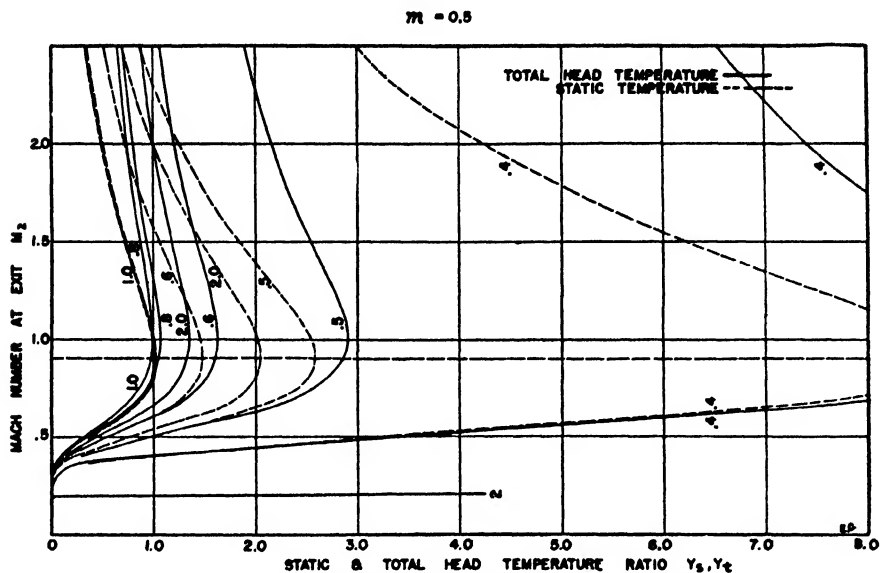


FIG. 5. Variation of the static and total head temperature ratio with the Mach number.

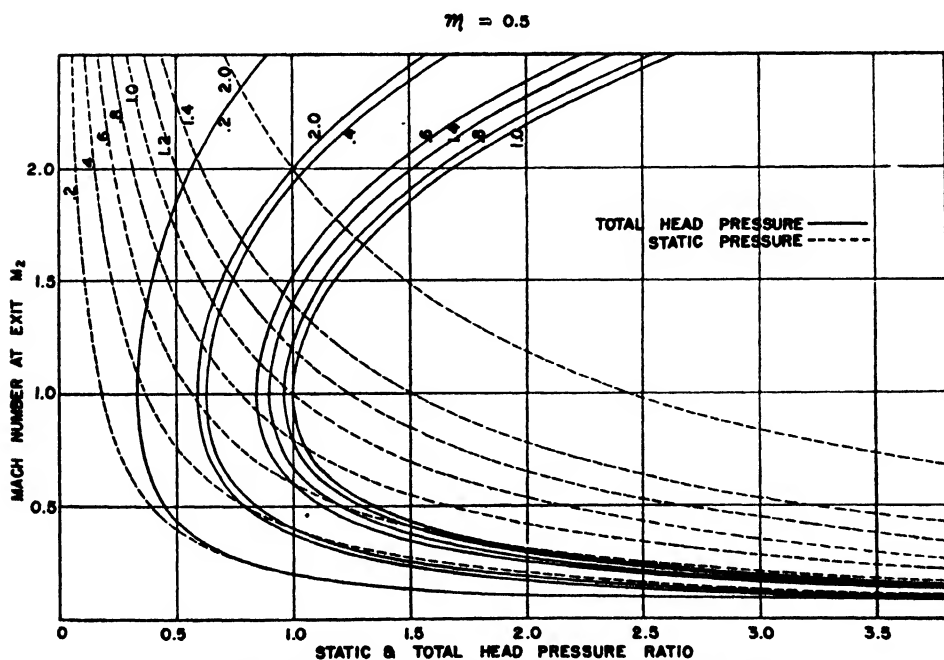


FIG. 6. Variation of the static and total head pressure ratio with the Mach number.

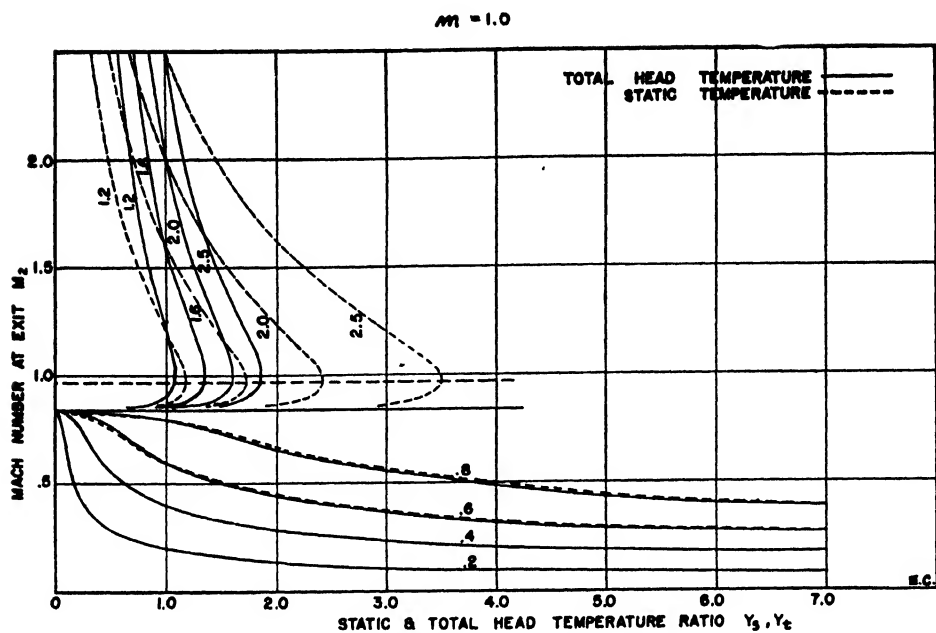


FIG. 7. Variation of the static and total head temperature ratio with the Mach number.

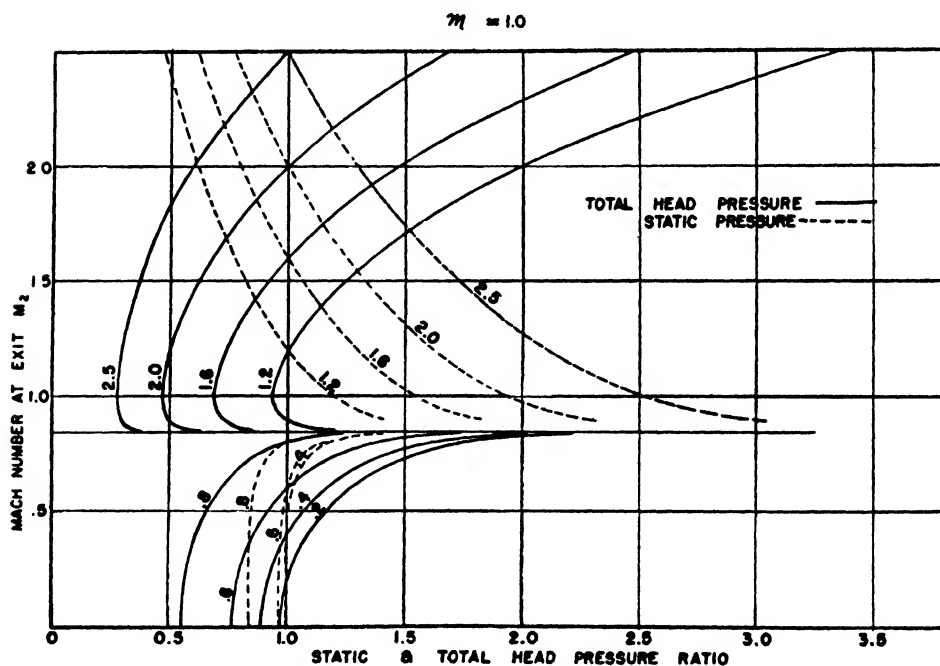


FIG. 8. Variation of the static and total head pressure ratio with the Mach number.

$$\eta = 1.5$$

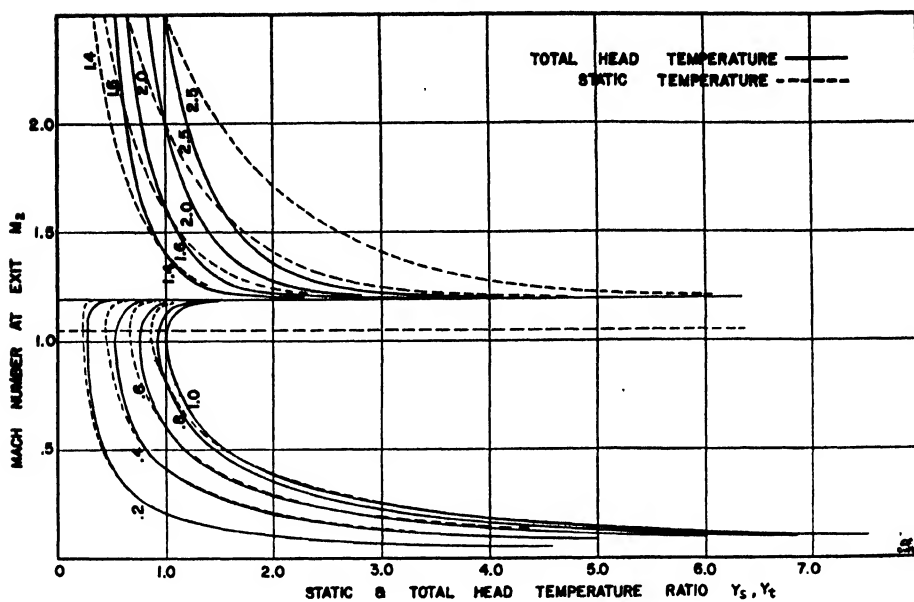


FIG. 9. Variation of the static and total head temperature ratio with the Mach number.

$$\eta = 1.5$$

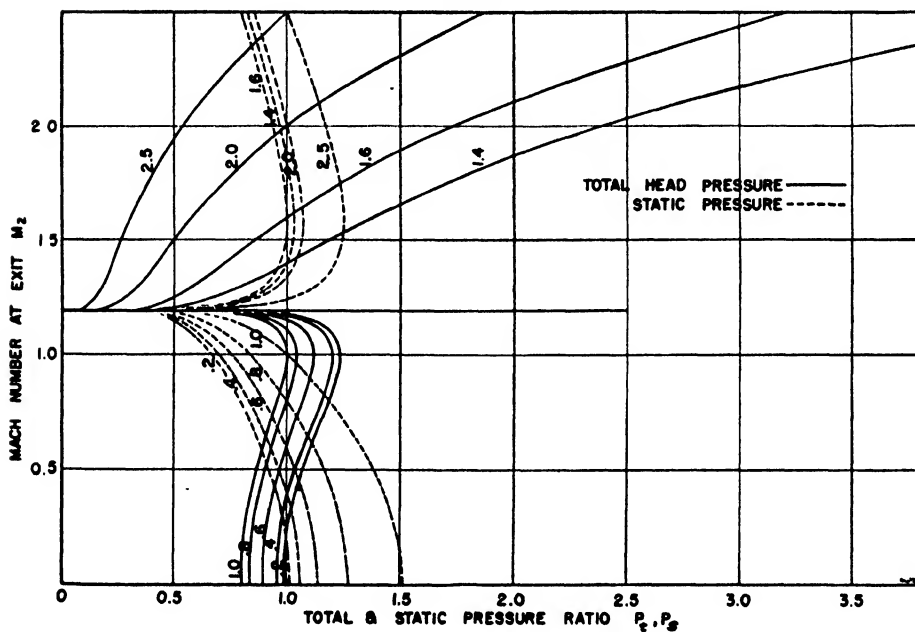


FIG. 10. Variation of the static and total head pressure ratio with the Mach number.

When $m < \frac{1+\gamma}{2}$ the maximum total head pressure loss occurs always when the Mach number equals unity. In the subsonic region the relative value increases with decreasing inlet Mach number, although in the supersonic region the opposite is true.

When $m > \frac{1+\gamma}{2}$ instead of total head pressure loss a total head pressure gain occurs and the maximum occurs when the Mach number equals unity; the reason for this is that the total head temperature decreases as the Mach number increases; therefore heat addition takes place only when the Mach number decreases.

5.0 Conclusions

From the above analysis and discussion it may be concluded that:

Heat can be added in a duct until the Mach number at a certain cross-section, most often the outlet, reaches unity.

In a duct, the total head temperature and pressure ratio reach an extreme value when the Mach number equals unity.

In the subsonic region the larger the divergence of the duct and the smaller the inlet Mach number, the larger is the extreme value of the total head temperature, i.e., the larger the amount of the heat acquired by the fluid.

In the supersonic region the larger the divergence of the duct and the larger the inlet Mach number, the larger is the extreme value of the total head temperature.

The static temperature reaches an extreme value for several values of the Mach number, smaller or larger than unity, depending on the divergence of the duct; but the static temperature does not represent the amount of the heat added in the fluid.

The friction losses result in a convergence effect on the duct. In the ideal case of a divergent duct with no friction losses, heat can be added without total head pressure loss if the Mach number is kept constant. In the real case a larger divergence must be used in order to compensate for the convergence effect of friction.

Appendix I

Derivation of the Mach Number Equation

From Equation (1), by substitution Equation (6) was found:

$$d \left\{ \rho_s V^2 A \left(1 + \frac{1}{\gamma Z} \right) \right\} - p_s dA + \frac{1}{2} C_f \rho_s \left(\frac{V}{\cos \theta} \right)^2 \cdot 2\pi r dx = 0, \quad (6)$$

and from Equations (3) and (4)

$$V d \left\{ \frac{V}{g} Q \left(1 + \frac{1}{\gamma Z} \right) - \frac{VR_c T_c}{1 + \frac{\gamma-1}{2} Z} \cdot g \rho_s dA + \frac{\rho_s V}{2} C_f \left(\frac{V}{\cos \theta} \right)^2 2\pi r dr \right\} = 0,$$

and substituting for $dA = 2\pi r dr$, $dr = dx \cdot t g \theta$ and differentiating:

$$V^2 d\left(\frac{1}{\gamma Z}\right) + \left(1 + \frac{1}{\gamma Z}\right) \frac{1}{2} dV^2 - g \frac{\rho_s V}{Q} \cdot \frac{g R_c T_i dA}{1 + \frac{\gamma-1}{2} Z} + \frac{g \rho_s V}{Q} C_f \frac{V^2 dA}{\sin 2\theta} = 0,$$

and

$$\begin{aligned} & -g\gamma R_c \frac{Z \cdot y_t dZ}{\gamma Z^2 \left(1 + \frac{\gamma-1}{2} Z\right)} + \frac{1}{2} \left(1 + \frac{1}{\gamma Z}\right) d \left\{ \frac{Z y_t}{1 + \frac{\gamma-1}{2} Z} \right\} g\gamma R_c \\ & - g\gamma R_c \left\{ \frac{y_t}{\gamma \left(1 + \frac{\gamma-1}{2} Z\right)} - \frac{C_f}{\sin 2\theta} \left(\frac{Z y_t}{1 + \frac{\gamma-1}{2} Z} \right) \right\} \frac{dA}{A} = 0 \\ & - \frac{y_t dZ}{\gamma Z \left(1 + \frac{\gamma-1}{2} Z\right)} + \frac{1}{2} \frac{1 + \gamma Z}{\gamma \left(1 + \frac{\gamma-1}{2} Z\right)} dy_t + \frac{1}{2} \frac{1 + \gamma Z}{\gamma Z} \\ & \cdot y_t \frac{dZ}{\left(1 + \frac{\gamma-1}{2} Z\right)^2} - y_t \frac{d \ln A}{\gamma \left(1 + \frac{\gamma-1}{2} Z\right)} \left(1 - \frac{\gamma Z C_f}{\sin 2\theta}\right) = 0 \\ & - \frac{y_t}{Z} \cdot \frac{dZ}{dy_t} + \frac{1}{2} (1 + \gamma Z) + \frac{y_t (1 + \gamma Z)}{2Z \left(1 + \frac{\gamma-1}{2} Z\right)} \cdot \frac{dZ}{dy_t} \\ & - y_t \left(1 - \frac{\gamma Z C_f}{\sin 2\theta}\right) \frac{d(\ln A)}{dy_t} = 0 \end{aligned}$$

and finally

$$\frac{1 - Z}{Z \left(1 + \frac{\gamma-1}{2} Z\right)} \cdot \frac{dZ}{dy_t} + 2 \left(1 - \frac{\gamma C_f Z}{\sin 2\theta}\right) \cdot \frac{d(\ln A)}{dy_t} = \frac{1 + \gamma Z}{y_t}. \quad (7)$$

Appendix II

Derivation of the Total Head Pressure Ratio Equation

From Equations (1) and (2) we obtain the following:

$$\begin{aligned} d \left\{ \frac{p_t (1 + \gamma Z) A}{\left(1 + \frac{\gamma-1}{2} Z\right)^{\left(\frac{\gamma}{\gamma-1}\right)}} \right\} & = \frac{p_t dA}{\left(1 + \frac{\gamma-1}{2} Z\right)^{\left(\frac{\gamma}{\gamma-1}\right)}} + \\ & \frac{C_f}{\sin 2\theta} \cdot \frac{\gamma p_t Z dA}{\left(1 + \frac{\gamma-1}{2} Z\right)^{\left(\frac{\gamma}{\gamma-1}\right)}} = 0, \end{aligned}$$

and

$$\frac{p_i(1 + \gamma Z)}{\left(1 + \frac{\gamma - 1}{2} Z\right)^{\left(\frac{\gamma}{\gamma-1}\right)}} + \frac{d}{d(\ln A)} \left\{ \frac{p_i(1 + \gamma Z)}{\left(1 + \frac{\gamma - 1}{2} Z\right)^{\left(\frac{\gamma}{\gamma-1}\right)}} \right\} \\ - \frac{p_i}{\left(1 + \frac{\gamma - 1}{2} Z\right)^{\left(\frac{\gamma}{\gamma-1}\right)}} + \frac{C_f}{\sin 2\theta} \frac{\gamma p_i Z}{\left(1 + \frac{\gamma - 1}{2} Z\right)^{\left(\frac{\gamma}{\gamma-1}\right)}} = 0,$$

and

$$\gamma p_i Z \frac{1 + \frac{C_f}{\sin 2\theta}}{\left(1 + \frac{\gamma - 1}{2} Z\right)^{\left(\frac{\gamma}{\gamma-1}\right)}} + \frac{d}{d(\ln A)} \left\{ \frac{p_i(1 + \gamma Z)}{\left(1 + \frac{\gamma - 1}{2} Z\right)^{\left(\frac{\gamma}{\gamma-1}\right)}} \right\} = 0,$$

but

$$d(\ln A) = m \frac{dy_i}{y_i} = \frac{m}{1 - 2m} \cdot \frac{(1 - Z)dZ}{Z \left(1 + \frac{\gamma - 1}{2} Z\right)(1 + \alpha Z)},$$

and substituting in the former equation we obtain:

$$\frac{\gamma m}{1 - 2m} \left(1 + \frac{C_f}{\sin 2\theta}\right) \frac{(1 - Z)dZ}{(1 + \alpha Z)(1 + \gamma Z) \left(1 + \frac{\gamma - 1}{2} Z\right)} \\ + d \ln \left\{ \frac{p_i(1 + \gamma Z)}{\left(1 + \frac{\gamma - 1}{2} Z\right)^{\left(\frac{\gamma}{\gamma-1}\right)}} \right\} = 0,$$

and after integration

$$Rel \left\{ \frac{p_i(1 + \gamma Z)}{\left(1 + \frac{\gamma - 1}{2} Z\right)^{\left(\frac{\gamma}{\gamma-1}\right)}} \right\} = Rel \left\{ \frac{\left(1 + \frac{\gamma - 1}{2} Z\right)^{(\gamma - \alpha)}}{(1 + \alpha Z)^{(1 + \alpha)}(1 + \gamma Z)^{(\gamma - 1 - \alpha)}} \right\}^{\frac{\frac{\gamma m}{1 - 2m} \left(1 + \frac{C_f}{\sin 2\theta}\right)}{(\gamma - \alpha) \left(\frac{\gamma - 1}{2} - \alpha\right)}}$$

and after some algebraic transformations we finally obtain

$$Rel p_i = Rel \left\{ \frac{\left(1 + \frac{\gamma - 1}{2} Z\right)^{\left(\frac{\gamma + 1}{\gamma - 1} \alpha\right)}}{(1 + \alpha Z)^{(1 + \alpha)}} \right\}^{\left(\frac{1}{\frac{\gamma - 1}{2} - \alpha} - \gamma\right)} \quad (21)$$

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PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

XII. ANTIFREEZE PROPERTIES OF TERNARY AQUEOUS SOLUTIONS CONTAINING *LEVO*-2,3-BUTANEDIOL AS A MAJOR COMPONENT¹

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Abstract

Freezing point, viscosity, and boiling point data are presented for aqueous solutions of *levo*-2,3-butanediol containing methanol, ethanol, ethylene glycol, and tetrahydrofurfuryl alcohol as third components. All four ternary systems show freezing points of -50°C . and lower over a considerable range of compositions. Among the compounds tested as third components, methanol was most effective as a thinning agent and accessory freezing point depressant. The data indicate that 20% methanol-40% butanediol-40% water is suitable for use at temperatures as low as -50°C .

Introduction

Judging from the variety of antifreeze commodities sold on the civilian market from year to year, the price of antifreeze contributes quite as much to its popularity as its physical properties. It is generally agreed that the properties of ethylene glycol render it particularly suitable for use as an antifreeze. On a basis of initial cost, this chemical has remained the most expensive of all established antifreeze compounds by a considerable margin. For this reason more than any other, it has not proved universally acceptable to motorists.

The war-time removal of ethylene glycol and glycerol from the home market led to a more general use of non-permanent antifreeze compounds by civilians. During this period, the production and properties of *levo*-2,3-butanediol have been studied intensively. Present indications are that this compound may be usefully employed as a non-volatile antifreeze (1, 8). When surplus agricultural products are employed as the fermentation substrate, it is considered possible to market this compound at a lower price than that of ethylene glycol either at the present time or at any period since it came on the market two decades ago (1).

It has already been shown that protection against freezing is provided down to -40°C . by 60 to 70% *levo*-2,3-butanediol solutions (1). In view of

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the high viscosity of concentrated butanediol solutions at low temperatures, the 50% solution was selected as most suitable for use in automobile radiators. This strength has been used in automobiles throughout the winters of 1944-45 at Ottawa and of 1945-46 at Ottawa and Saskatoon. By correcting evaporation losses with water throughout the winter, adequate protection was provided, as with other permanent-type compounds. A practical device, based on the relation of flow-time to concentration and temperature, has been provided for the testing of butanediol solutions after use in radiators (9).

Reference has already been made in the technical literature to the usefulness of ternary antifreeze solutions and a number of patents have been granted for radiator fluids of this type (10). Freezing point data have been provided for the systems methanol-glycerol-water and methanol-ethylene-glycol-water by Feldman and Dahlstrom (6) and Conrad, Hill, and Ballman (3), respectively. Viscosity data for ethanol-glycerol-water solutions at 25° C. have also been presented by Ernst, Watkins, and Ruwe (5). The advantages claimed include lowering of the cost and viscosity by the alcohol, and lowering of volatilization losses by the non-volatile component; thus the advantages of each agent are retained to some extent while the objectionable characteristics are minimized.

It must be borne in mind, however, that the third component alters the physical properties that provide the basis for practical tests of freezing point protection in binary aqueous solutions. If the third component is volatile, it is also necessary to add this component as well as water in correcting volume losses.

The purpose of the present investigation was the provision of radiator solutions suitable for use in extremely cold weather (-45° to -50° C.) by blending aqueous *levo*-2,3-butanediol* solutions with other antifreeze chemicals. Methanol, ethanol, ethylene glycol, and tetrahydrofurfuryl alcohol were selected for study as third components, and the suitability of the various combinations was assessed by measurements of freezing point, viscosity, and boiling point.

Materials and Methods

Tetrahydrofurfuryl alcohol (Eastman Kodak) was purified by distillation through a Stedman column, and the fraction having a refractive index of 1.4505 at 25° C. was retained for use. Preparation of the remaining chemicals and methods employed in determining freezing point, viscosity, and boiling point have already been described (1).

Data for the ternary diagrams were derived from sets of curves for two- and three-component series. The viscosity data plotted on triangular coordinates are based on a smaller number of determinations than the freezing point figures and should therefore be regarded as approximations. They are considered sufficiently accurate to establish the general tendencies under discussion.

* Hereafter, the term "butanediol" will be understood to refer to *levo*-2,3-butanediol.

Results and Discussion

METHANOL-BUTANEDIOL-WATER

Freezing Point

The outstanding features of the freezing point diagram for this system arise from the effectiveness of methanol as an accessory antifreeze, the range of compositions capable of withstanding -50°C . being much wider than in any other system studied (Fig. 1). The ternary solution containing 50% water and 15% methanol provides ample protection against freezing to temperatures of -40°C . With water contents of 20 to 40% and a methanol content of 10%, the freezing point is below -45°C . 5% Methanol-60% butanediol-35% water freezes at approximately -50°C . The curvature of the upper tie-lines indicates lower freezing points for ternary systems of high water content than would be expected from strictly additive effects of the two freezing point depressants. There is earlier evidence of this latter property in the freezing point data of methanol-glycerol-water (6) and some indications of it in the system methanol-ethylene-glycol-water, at least for compositions freezing at -40° to -50°C . (3).

Viscosity

Interpretation of Fig. 2 is considerably facilitated by assuming that methanol and butanediol undergo hydration (1, 7). Within series of compositions containing 40% or more of water, and in which butanediol is present in constant amount, replacement of part of the water with methanol does not reduce the viscosity appreciably. The viscosities of 40% butanediol-60% water, and 20% methanol-40% butanediol-40% water, for instance, are practically identical.

The complications introduced by hydration are well illustrated by the pronounced curvature of the 2.5-centistoke tie-line, which includes compositions containing 20% butanediol at widely different water contents. Beginning with 20% butanediol-80% water, substitution of methanol for water at first increases the viscosity. As the proportion of methanol is further increased, the viscosity decreases. The present data signify that considerable reduction in viscosity is effected by substitution of methanol for part of the butanediol at uniform water contents, e.g., 40%. This change results from the difference in viscosity of hydrated methanol and butanediol, which difference is less pronounced than that shown by the anhydrous chemicals.

At 20°C . the viscosities in centistoke units for 40% butanediol-60% water, 60% ethylene glycol-40% water, and 20% methanol-40% butanediol-40% water are 5.24, 4.71, and 5.17, respectively, or approximately one-half that of 60% butanediol-40% water at the same temperature (10.8 centistokes). It has already been shown that 50% butanediol is the most suitable concentration for use as a binary aqueous antifreeze, higher concentrations being undesirably viscous, and lower concentrations having freezing points above -30°C . (1). On mixing 50% aqueous butanediol solution with methanol in the ratio of 4 : 1 by weight to obtain the ternary solution 20% methanol-40%

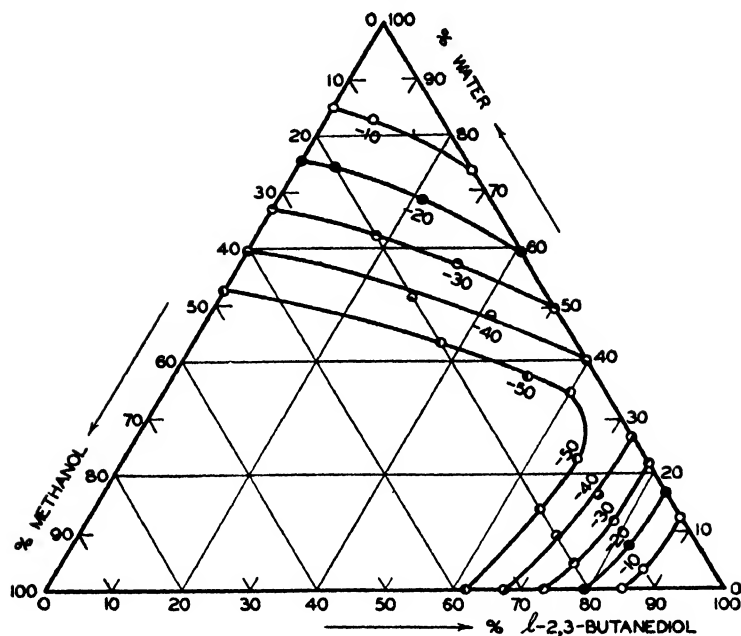


FIG. 1. Freezing points of aqueous *l*-2,3-butanediol-methanol solutions.

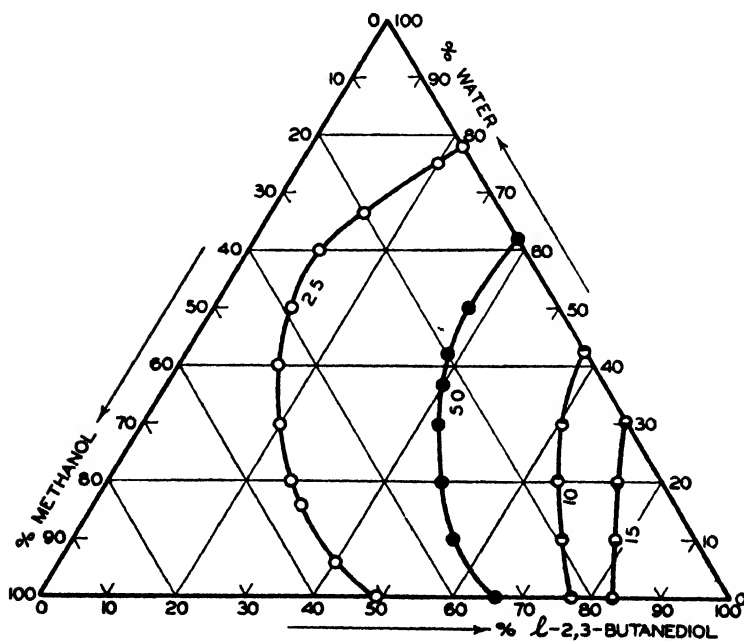


FIG. 2. Kinematic viscosity of aqueous *l*-2,3-butanediol-methanol solutions at 20° C., expressed in centistokes.

butanediol-40% water, the freezing point is reduced to -50°C . and the viscosity at 20°C . is reduced to less than half that of 60% butanediol-40% water and almost to that of 60% ethylene glycol-40% water.

Boiling Point

The boiling point of methanol-butanediol-water solutions is strongly influenced by methanol content (Fig. 3). Over the range 30 to 50%, water

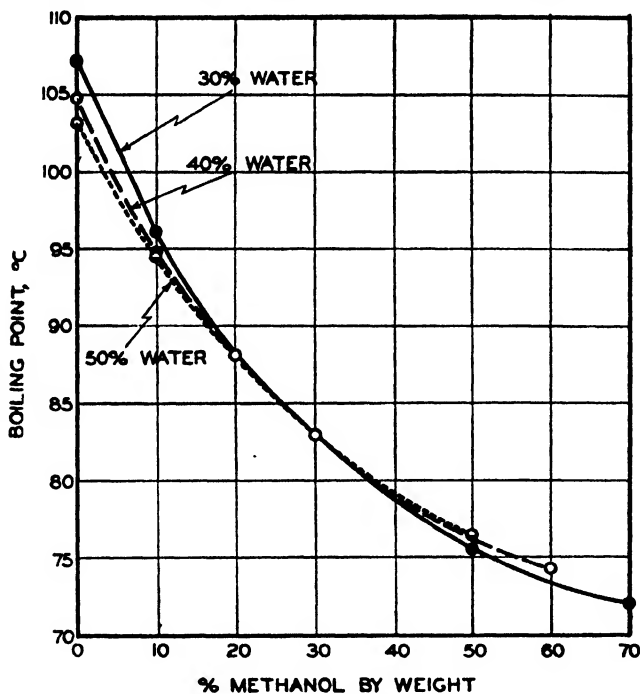


FIG. 3. Boiling points of aqueous 1,2,3-butanediol-methanol solutions.

content has little effect on the boiling point of systems having identical methanol contents. The boiling points of the ternary systems are slightly higher than those of binary aqueous solutions of the same methanol content: e.g. 20% methanol-40% butanediol-40% water, 88.2°C .; 20% methanol-80% water, 86.2°C . The boiling points of the ternary systems containing methanol (Table I) are substantially higher than that of 50% aqueous methanol solution.

TABLE I
FREEZING POINT AND BOILING POINT DATA FOR BINARY AND
TERNARY AQUEOUS METHANOL SOLUTIONS

Composition	F.p., $^{\circ}\text{C}$.	B.p., $^{\circ}\text{C}$.
10% methanol-50% butanediol-40% water	-48	94.7
10% methanol-90% water	-6	91.7
20% methanol-40% butanediol-40% water	Below -50	88.2
20% methanol-80% water	-15	86.2
50% methanol-50% water	Below -50	76.5

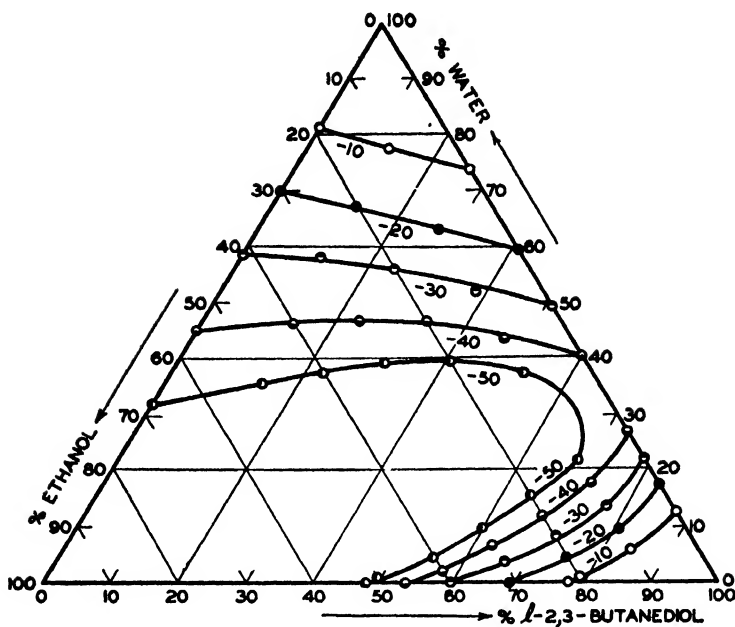


FIG. 4. Freezing points of aqueous l-2,3-butanediol-ethanol solutions.

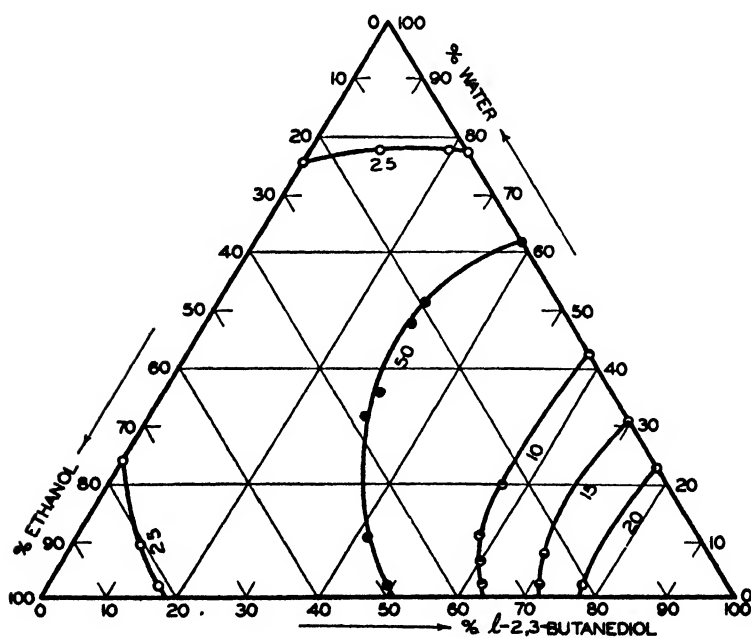


FIG. 5. Kinematic viscosity of aqueous l-2,3-butanediol-ethanol solutions at 20°C., expressed in centistokes.

ETHANOL-BUTANEDIOL-WATER

Freezing Point

Fig. 4 shows that ethanol is less effective than methanol as a supplementary freezing-point depressant. With 30% water, protection to -50°C . is provided by the presence of 10% ethanol. A slightly higher freezing point is shown by the system 20% ethanol-40% butanediol-40% water, which has the advantage of higher water content and fluidity. Apparently there are no ternary ethanol systems of the present type that have freezing points below -50°C . when the water content is 40% or higher. Larger amounts of ethanol are required in reducing the freezing point of the anhydrous diol to a standard extent than with methanol, as would be expected from their molecular weights.

Viscosity

Considerable similarity is shown in the viscosity diagrams for the ternary methanol and ethanol systems (Figs. 2, 5), the principal difference being the

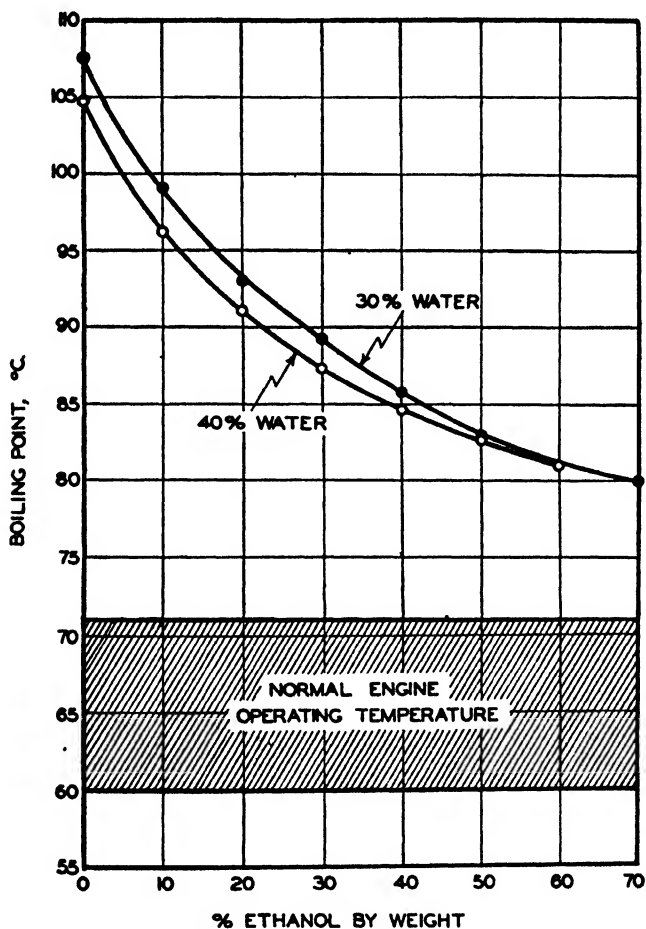


FIG. 6. Boiling points of aqueous 1-2,3-butanediol-ethanol solutions.

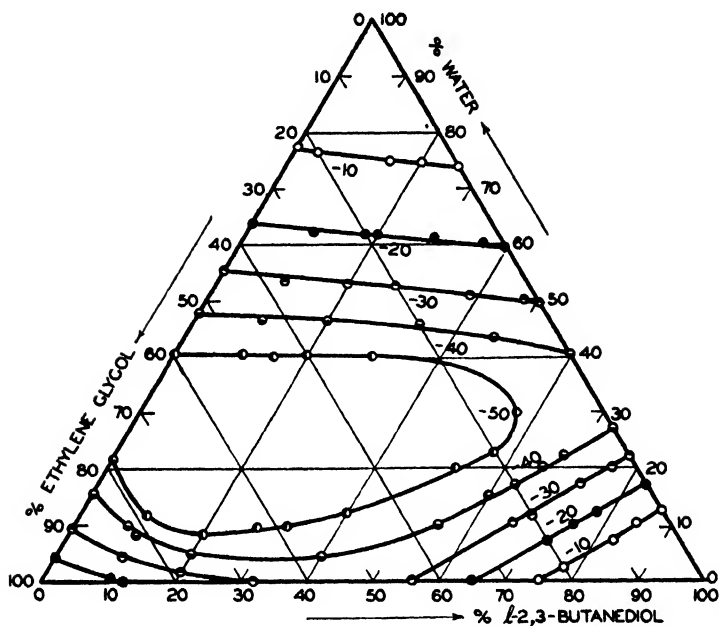


FIG. 7. Freezing points of aqueous *l*-2,3-butanediol-ethylene-glycol solutions.

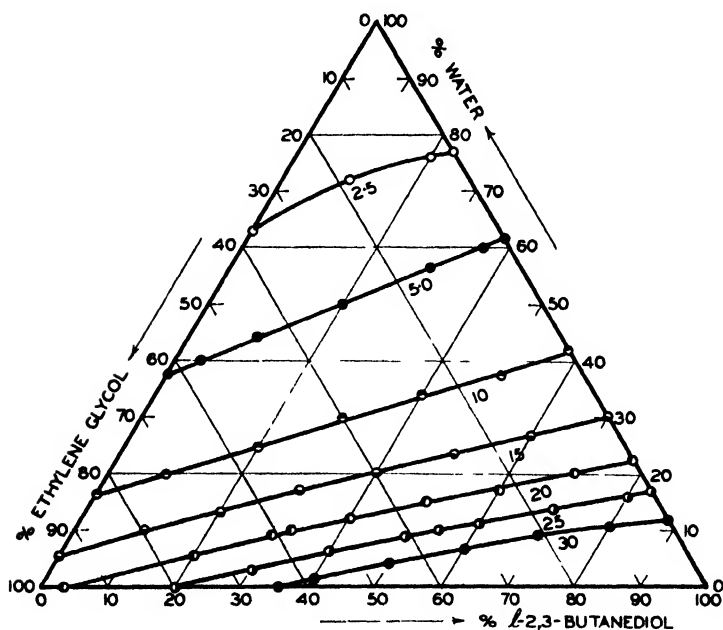


FIG. 8. Kinematic viscosity of aqueous *l*-2,3-butanediol-ethylene-glycol solutions at 20° C., expressed in centistokes.

consistently higher viscosity values shown in ethanol systems at equivalent percentages by weight. General agreement is also shown between the data of Fig. 5 and the viscosity data of Ernst, Watkins, and Ruwe for ethanol-glycerol-water at 25° C. (5). Aqueous solutions of ethanol-butanediol having a viscosity of 5 centistokes at 20° C. and a freezing point of -50° C. were characterized by water contents below 40%, and by approximately equal proportions of ethanol and butanediol.

Boiling Point

Aqueous ethanol solutions of low water content have higher boiling points than methanol solutions of corresponding concentration (4). There is little difference between the two ternary systems in this respect, however, so long as the total alcohol content does not exceed 20% (Figs. 3, 6). The present data provide no evidence of azeotropic mixtures of high boiling point in either of these ternary systems—addition of methanol or ethanol in appreciable amounts reduces the boiling point below 100° C. The ternary ethanol solutions have higher boiling points than are shown by binary aqueous solutions of the same ethanol content. The difference in boiling point of ternary and binary ethanol solutions of the same freezing point are particularly noteworthy: 10% ethanol-50% butanediol-40% water (f.p., -45° C.) boils at 96.3° C. while 65% ethanol, having the same freezing point, boils at 80.5° C.; 20% ethanol-40% butanediol-40% water, which has approximately the same freezing point as the foregoing solutions, boils at 91.2° C.

ETHYLENE-GLYCOL-BUTANEDIOL-WATER

Freezing Point

For systems containing 50% and higher percentages of water, ethylene glycol is less effective than ethanol as an accessory freezing point depressant (Figs. 4, 7). The -40° C. tie-line, however, passes through points representing almost identical compositions in these two diagrams. With water contents of 20 to 40%, reduction of the freezing point to -50° C. requires larger percentages of ethylene glycol than of either methanol or ethanol: at 40% water, 30% ethylene glycol is required; with 30% water, 15% ethylene glycol suffices (Fig. 7). In the absence of water, the minimal freezing point value of the mixed diols is approximately -35° C.

Viscosity

The outstanding feature of the viscosity diagram (Fig. 8) is the regularity with which this property is altered with changing proportions of the two freezing point depressants, regardless of the water content. The data show that the viscosity of aqueous butanediol solutions cannot be reduced to any great extent by small additions of ethylene glycol, e.g., 10 to 20%.

Figs. 9 and 10 show the effect of butanediol and ethylene glycol contents on the low temperature viscosities of systems containing 40 and 30% water, respectively. With 40% water the viscosity at -50° C. rises sharply with decreasing ethylene glycol content, exceeding 1000 centistokes at a butanediol content of 12.5%. At -40° C., the 40% water series shows a viscosity of

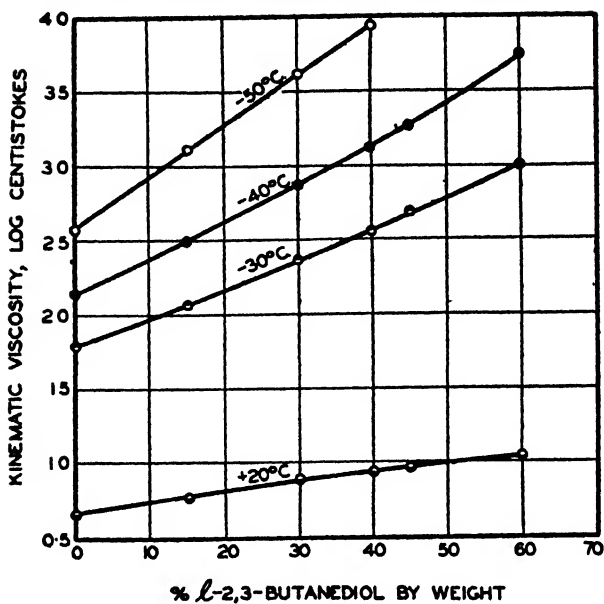


FIG. 9. Kinematic viscosity at low temperatures of l-2,3-butanediol-ethylene-glycol solutions containing 40% water.

1000 centistokes at a butanediol content of 35% (Fig. 9), whereas the 30% water series shows a viscosity of 1000 centistokes at a butanediol content of 30% (Fig. 10).

Boiling Point

The boiling point of this system is strongly affected by water content, as in binary solutions of either diol. When the water content is maintained constant at levels of 40% and higher, the boiling point is not changed appreciably by different proportions of the two freezing point depressants, the same applying to the system, tetrahydrofurfuryl-alcohol-butanediol-water.

TETRAHYDROFURFURYL-ALCOHOL-BUTANEDIOL-WATER

Freezing Point

The range of compositions freezing at and below -50°C . is more restricted in this ternary system (Fig. 11) than in those considered previously, for practical purposes being limited to water contents of 30% or less. It is in essentially anhydrous systems that this alcohol shows particular promise as an antifreeze (2).

Viscosity

Aqueous solutions of tetrahydrofurfuryl alcohol are less viscous than aqueous butanediol solutions of corresponding concentration, and the ternary mixtures in general show intermediate values (Fig. 12). The curvatures of the 10- and 15-centistoke tie-lines are attributed to hydration effects operating in the

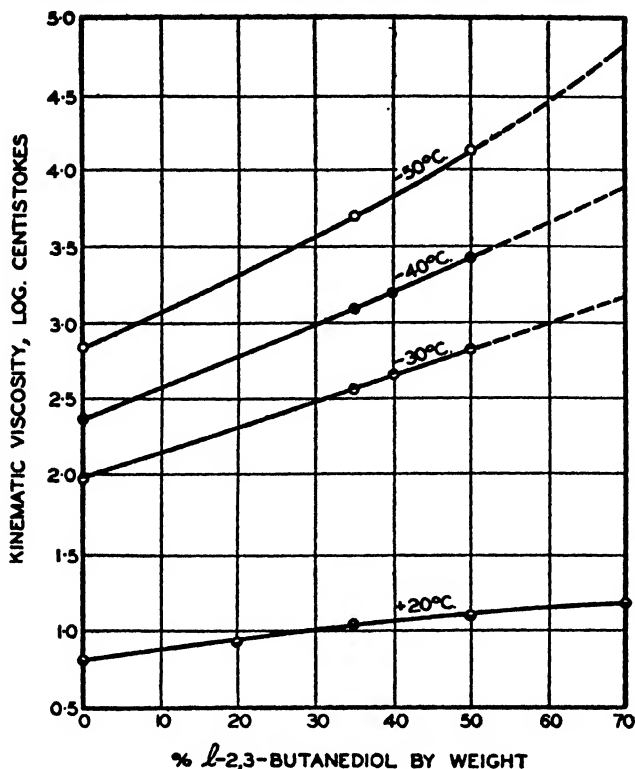


FIG. 10. Kinematic viscosity at low temperatures of l-2,3-butanediol-ethylene-glycol solutions containing 30% water.

same manner as in ternary methanol and ethanol systems (Figs. 2, 5). The strong curvature of the 10-centistoke line appears at a relatively low water content. This effect of hydration at low concentrations of water is also apparent in the absence of butanediol: aqueous tetrahydrofurfuryl alcohol solutions show maximal density and viscosity values at water contents of 10 to 20% (2).

Viscosity measurements were made at low temperatures on several aqueous solutions of tetrahydrofurfuryl-alcohol-butanediol freezing at or below -50°C . The values were very similar, however, to those shown by 60% butanediol at the same temperatures.

Conclusions

Among the third components dealt with in this paper, methanol is judged most effective as an accessory freezing point depressant and thinning agent. As little as 5% methanol suffices to reduce the freezing point to -50°C . Greater amounts of methanol are required in reducing the viscosity to that of 60% aqueous ethylene glycol solution, and use of methanol in substantial amounts lowers the boiling point.

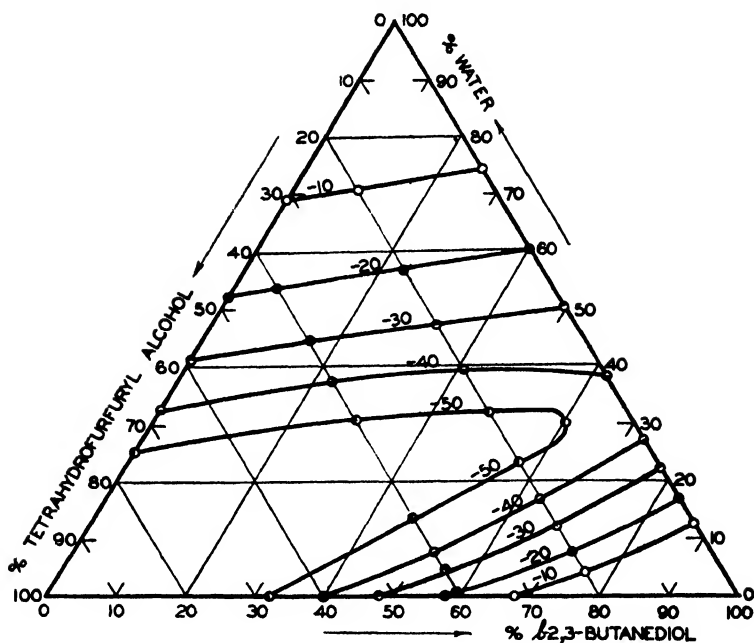


FIG. 11. Freezing points of aqueous *l*-2,3-butanediol-tetrahydrofurfuryl-alcohol solutions.

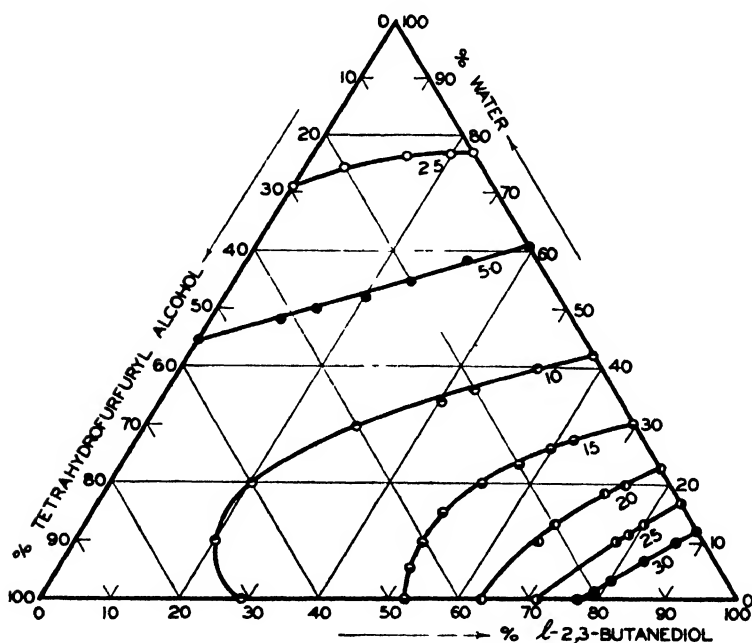


FIG. 12. Kinematic viscosity of aqueous *l*-2,3-butanediol-tetrahydrofurfuryl-alcohol solutions at 20°C., expressed in centistokes.

The ternary solution 20% methanol-40% butanediol-40% water does not freeze at $-50^{\circ}\text{C}.$, has a considerably higher boiling point than aqueous methanol solutions of equivalent freezing point, and is almost as fluid as 60% ethylene glycol at $20^{\circ}\text{C}.$ On the basis of laboratory tests, this ternary mixture appears suitable for use at temperatures as low as -45° to $-50^{\circ}\text{C}.$ Its practical value as a radiator solution for use under these extreme conditions cannot be fully assessed until it has been exposed to similar conditions in engine cooling systems.

Acknowledgments

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PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

XIII. PURIFICATION OF BY-PRODUCT ETHANOL¹BY E. L. TOLLEFSON², J. A. WHEAT³, AND J. D. LESLIE³

Abstract

Samples of high wines recovered from three whole wheat mashes fermented by *Aerobacillus polymyxa* were fractionated by rectification. It was shown that the high wine from this fermentation resembles that obtained from a yeast fermentation and that a satisfactory industrial ethanol may be prepared by distillation methods. By preparation of derivatives, acetone, diacetyl, and acetic acid were shown to be present in the high wine.

Introduction

Fermentation of carbohydrates by *Aerobacillus polymyxa* produces *levo*-2,3-butanediol as the principal product of commercial value. In addition, a smaller amount of ethanol is produced, which in commercial practice would be of sufficient value to justify recovery. Yields, ratio of diol to ethanol, and other characteristics of the fermentation have been discussed in previous papers of this series (2, 7, 13). The pilot plant process for both fermentation and recovery of products from fermented mash, as developed in these laboratories, has also been described (8, 9).

Ethanol is separated as high wine in the first recovery operation, which consists of stripping fermented mash, at atmospheric pressure, in a conventional type of beer column. The overhead, or distillate, is completely condensed, part being removed as high wine and the remainder returning to the column as reflux. The stripped mash, or slops, is discharged from the bottom of the column.

The overhead product contains 60 to 80% by volume of ethanol, as well as small amounts of several other substances that must be partially or completely removed if the ethanol is to meet specifications for industrial use. These impurities are formed mainly during the fermentation, but it is conceivable that some may result from chemical reactions taking place during the stripping operation.

Batch rectification of high wine in the pilot plant had shown that impurities occurred in the fractions distilled off both before and after the ethanol fraction; in particular a two phase material appeared between the ethanol and water fractions. This indicated that the material contains impurities analogous to those present in the high wine recovered from a yeast fermentation. In this industry high wine is separated by rectification into four fractions: aldehydes,

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ethanol, fusel oil, and water. This separation may be carried out on either a continuous or batch basis and usually yields a pure ethanol-water product of approximately the azeotropic composition. For continuous operation, two (10) or three (4) distillation columns are required, whereas for batch operation a still pot and one column suffice.

The purpose of the present work was (i) to determine the nature and amounts of the impurities in the high wine, and (ii) to investigate the conditions for obtaining a satisfactory industrial ethanol.

Methods and Materials

The general method of investigation was to fractionate high wine by batch distillation under carefully controlled conditions, and to analyse the product fractions for various components as described below. Some of the fractions were again rectified in a smaller apparatus, and individual compounds were identified by boiling points, refractive indices, and preparation of derivatives. Samples of high wine recovered from three fermentations were fractionated. The fermentation conditions differed in that in the first run half of the mash was aerated with 2.6 c.f.m. per 100 gal. for 48 hr., in the second the rate of aeration was the same but was continued for 72 hr., while the third was not aerated. All fermentations showed only a slight trace of contamination and all gave efficiencies of over 90%.

Rectification was carried out in an iron column, 3 in. in diameter by 2 ft. high, packed with 24 stainless steel Stedman plates, and heavily lagged with asbestos. The column was mounted on a jacketed; steam heated, copper, still pot of 16 litres capacity. Into the top of the column was sealed a glass distilling head (shown in Fig. 1) to permit the setting and measurement of any desired reflux ratio. Two 50 cm. Liebig condensers provided ample condensing capacity. All fractionations were carried out at atmospheric pressure and observed boiling points were corrected to 760 mm.

Reflux ratios were measured as follows. The column was first brought to equilibrium under total reflux with stopcock x open and stopcock y closed. Then x was closed and the time, t_1 , for the cup to fill to level B was measured with a stopwatch. From this measurement and the known volume of the cup between levels A and B , the total condensate per minute could be computed. After draining the cup through x , y was opened and adjusted to give the desired take-off. Next, to determine the reflux ratio, x was again closed and the time, t_2 , for the cup to fill as before was measured. The ratio of reflux to take-off is given by the expression $t_1/(t_2 - t_1)$. A slight error is introduced by the increased flow through y when the level in the cup rises from A to B , but the effect was not important in this work. During operation of the column, stopcock x was left open and the product was drawn off through y .

In Run 1, 8 kgm. was charged to the still pot, heated to boiling and the column operated at total reflux until a constant top temperature indicated that equilibrium had been established. Then the take-off stopcock (y in

Fig. 1) was opened and distillate collected at an initial reflux ratio of 12. While the top temperature was rising, samples large enough for complete analysis were taken, each of which represented the average composition over a range of temperature.

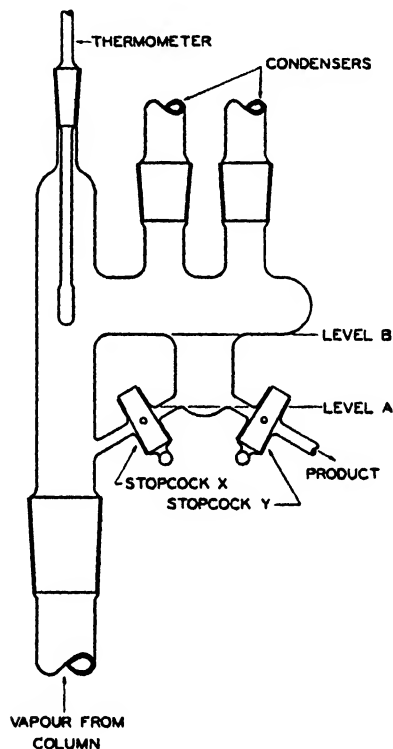


FIG. 1. Distillation head used in rectification of high wine.

When the top temperature reached 78.2°C ., indicating a composition corresponding to the ethanol-water azeotrope, the reflux ratio was decreased to 5 by increasing the rate of distillate take-off. The rate of boiling in the still pot was kept as constant as possible throughout the entire rectification. After removal of the main ethanol fraction in approximately 500 ml. lots, the temperature again rose, and at this point the reflux ratio was increased to 7. This value was maintained until the temperature reached 100.0°C ., showing that the distillate was water. When the amount of material left in the still became so small that the rate of boiling could not be maintained, rectification was stopped, and the column holdup was allowed to drain back to the pot. This material constituted the last sample.

The procedure for Run 2 was the same as that described above except that the charge was 10 kgm. and a reflux ratio of 10 was used at temperatures below 78.2°C ., and one of 2 above 78.2°C . In Run 3 a charge of 10 kgm. was distilled at an initial reflux ratio of 25. Then, when the temperature

reached 78.2° C. this was reduced to 1. When the temperature began rising after the removal of the ethanol fraction the reflux ratio was increased to 30.

All samples were analysed for titratable acidity, esters, fusel oil, and aldehydes, and in the third fractionation several samples were analysed for ethanol. These analyses were chosen since they are the basis for industrial ethanol specifications. The analytical procedures were as follows.

Acids.—A 25 gm. sample was titrated with 0.1 *N* sodium hydroxide to a phenolphthalein end-point. Results are reported as milliequivalents per 100 gm. of distillate.

Esters.—Excess 0.1 *N* sodium hydroxide was added to the previously neutralized sample, the mixture was shaken, allowed to stand overnight, and then was back titrated with 0.1 *N* sulphuric acid. The difference between the base added and that neutralized by acid was taken as the amount required to saponify the esters, and the results are expressed as milliequivalents per 100 gm. of distillate.

*Fusel oil.**—This analysis is a colorimetric one for the determination of the fusel oil content of industrial ethanol. Since it is not specific for fusel oil and can be used only for low concentrations it was applied only to samples of the ethanol fraction. The reagent is prepared by mixing 1 gm. of salicylaldehyde with 100 ml. of 85% fusel-oil-free, ethanol. To 1.0 ml. of sample, 0.1 ml. of reagent and then 2 ml. of concentrated sulphuric acid was added in such a way as to produce layering. The mixture was shaken vigorously and allowed to stand for five minutes. Colours ranging from lemon yellow, through orange, to red indicated 5 to 30 mgm. of fusel oil per 100 ml. of sample. By diluting the original sample, the range of fusel oil content was extended to 150 mgm. per 100 ml.

*Aldehydes.**—Silver ammonium hydroxide reagent was prepared by adding 1 ml. of 8.25% sodium hydroxide to 1 ml. of 8.25% silver nitrate, and then ammonium hydroxide (specific gravity 0.90) until the precipitate just dissolved. One millilitre of the freshly prepared reagent was added to 10 ml. of sample, and, after shaking, the mixture was allowed to stand in the dark for one hour. Formation of a white precipitate upon addition of 1 ml. of 10% sodium chloride solution indicated the presence of unreduced silver, and a trace of cloudiness satisfied the specification.

Ethanol.—The sample to be analysed was first diluted to give a solution containing less than 3.6% ethanol. Twenty-five millilitres of this solution was added to 25 ml. of water and four drops of 40% sodium hydroxide in a 500 ml. round bottom flask. About 35 ml. was distilled at constant volume, and collected in a 50 ml. volumetric flask. The distillate was made up to 50 ml. and 2.0 ml. of this added to 15 ml. of 0.2 *N* potassium dichromate and 25 ml. of 50% sulphuric acid in a pressure bottle. The pressure bottle was capped and placed in a bath of boiling water for 20 min. After cooling, 3.5 ml. of 30% potassium iodide was added and the liberated iodine titrated

* Unpublished methods from Joseph E. Seagram and Sons, Inc.

with 0.2 *N* sodium thiosulphate using starch as an indicator. The concentration of ethanol in the original sample was calculated from the difference between blank and sample titrations. Since aldehydes interfere in this determination they were removed prior to the distillation step by precipitation with 2,4-dinitrophenylhydrazine.

Experimental Results

Rectification of High Wines

The important characteristics of the three high wines that were fractionated are shown in Table I. The densities at 20° C. were calculated from specific gravities determined with a Westphal balance. The difference in acid and ester contents are believed to be attributable to the conditions of fermentation. Since it is known (1) that aeration of fermenting mash increases the production of organic acids and decreases ethanol formation the results in Table I are not unexpected.

TABLE I
CHARACTERISTICS OF THE HIGH WINES USED FOR FRACTIONATION

Run No.	Ethanol, % by weight	Density, gm./ml., 20° C.	Acid, m.e./100 gm.	Ester, m.e./100 gm.
1	66.9	0.8672	0.23	1.92
2	65.7	0.8677	0.79	2.95
3	66.6	0.8697	0.17	1.01

The change in boiling point throughout the distillations and the results of the analyses for acids and esters of the various fractions are shown in Figs. 2, 3, and 4. In Fig. 4 the ethanol concentration is also shown. In the range 4 to 68% distilled this was estimated from specific gravities determined with a Westphal balance and the other values are the results of chemical analyses of samples treated with 2,4-dinitrophenylhydrazine.

In Table II the mean acid, ester, and fusel oil contents are shown for the samples that had a satisfactory aldehyde content. A comparison of these results with the corresponding values for the original high wines (Table I) shows that strict proportionality does not hold. The discrepancies are attributed to differences in the reflux ratios and to possible variations in the chemical composition of the high wines. An additional fact, not shown in Table II, is that the ethanol fractions in Runs 1 and 2 had a trace of yellow colour; whereas this was not observed in Run 3. The yellow colour is known to be due to the presence of diacetyl, and is discussed further in the next section.

Identification of Components in High Wine Fractions

The boiling point curve for Run 2 indicated a considerable amount of some material boiling at 55 to 58° C. In order to identify this compound the fractions collected in the temperature range 52.8 to 58° C. were mixed and

TABLE II

CHARACTERISTICS OF THE ETHANOL FRACTION THAT MET ALDEHYDE SPECIFICATIONS

Run No.	Acid, m.e./100 gm.	Ester, m.e./100 gm.	Fusel oil, mgm./100 ml.	Distillation range, %
1	0.05	1.0	24	11.0-70.5
2	0.40	1.6	35	14.5-69.5
3	0.04	0.1	15	6.9-67.5

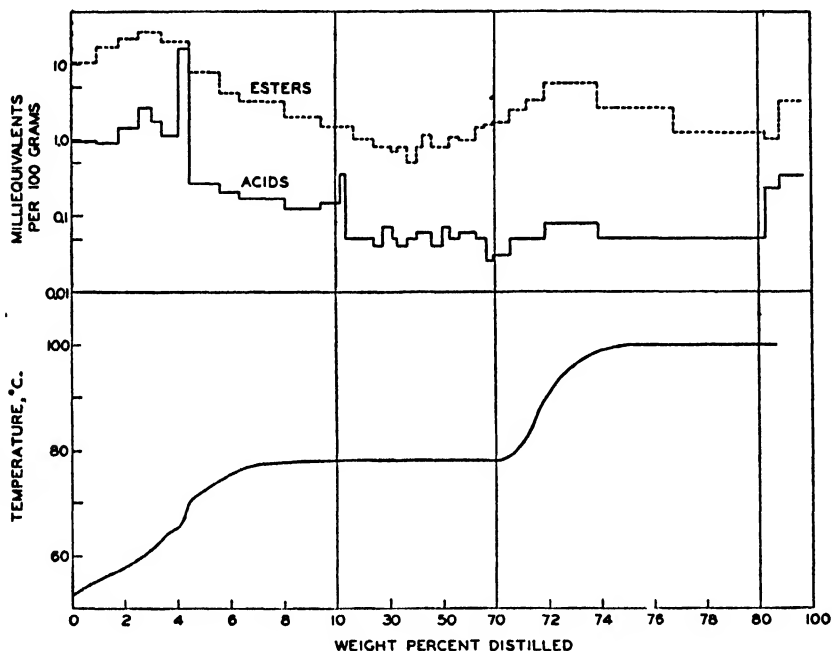


FIG. 2. Distillation curves of Run 1.

refractionated in a small Stedman column. A boiling point of approximately 55° C., maintained throughout the main part of the distillation, was a good indication that acetone was present. Refractive indices of the different fractions indicated that about 65% of the material redistilled was acetone. This corresponded to about 2% of the high wine. To prove that the product was acetone the 2,4-dinitrophenylhydrazine derivative was prepared. It gave a melting point of 126° C., which is the value given in the literature (12) for acetone 2,4-dinitrophenylhydrazone. A mixed melting point with an authentic derivative showed no deviation from this temperature, thus proving that the compound was acetone.

Since the odour of the residual liquid from the above redistillation suggested the presence of acetic acid the *p*-bromophenacyl ester was prepared from the neutralized residue and *p*-bromophenacyl bromide. After purification by

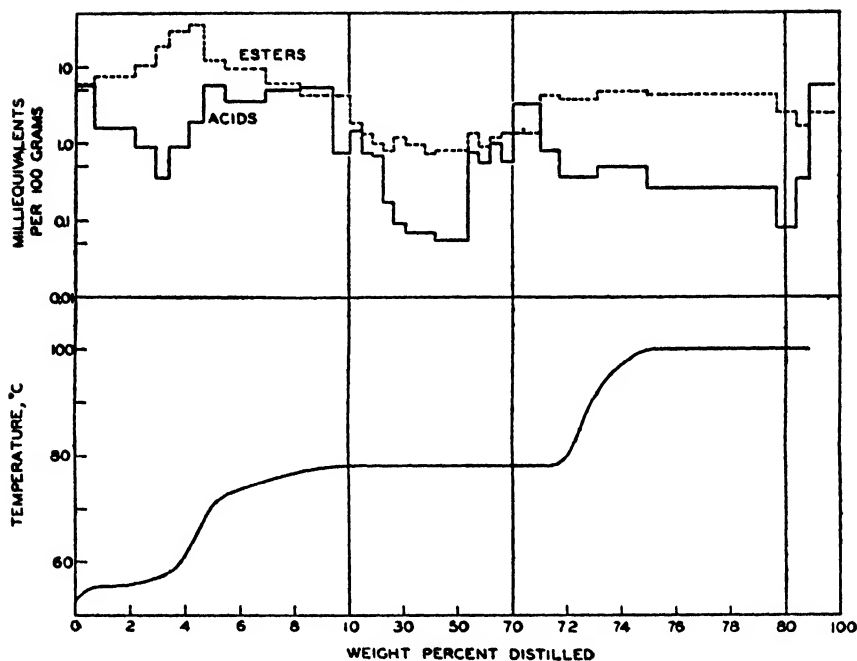


FIG. 3. Distillation curves of Run 2.

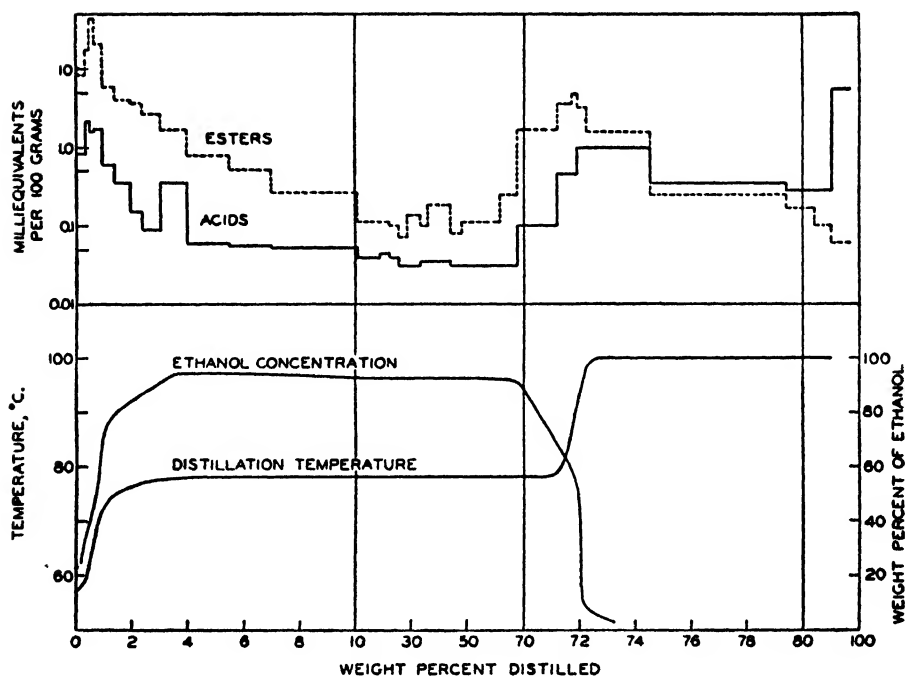


FIG. 4. Distillation curves of Run 3.

recrystallization, comparison of this derivative with an authentic derivative proved that acetic acid was present.

The fraction of high wine collected from 58° to 68° C. had the characteristic odour of esters. For this reason a saponification was carried out by adding sodium hydroxide solution and allowing the mixture to stand overnight. This was followed by a determination of the volatile fatty acids by the method of Hillig and Knudsen (5). When the required distillation had been completed the formic acid present was determined by Lazzari's method (10), although formic acid had not been identified. The result showed the presence of only a small amount. The volatile acid analysis proved that acetic was the main acid constituent.

Throughout the complete series of fractions collected in Runs 1 and 2 a yellow colour was observed. This was thought to be due to diacetyl and accordingly a phenylhydrazine derivative was prepared. The purified, crystalline product had the same melting point as an authentic sample of diacetyl bisphenylhydrazone. A mixed melting point, showing no deviation from this temperature, proved the presence of diacetyl. It was then possible to devise a method for purifying the ethanol. Enough sodium hydroxide was added, in pellet form, to neutralize the acids, saponify the esters, and polymerize the diacetyl. When these reactions were complete a simple distillation gave a colourless product of negligible acid and ester content. The values given in Table II were obtained before treatment with sodium hydroxide.

Following the removal of the ethanol fraction in the main rectification there was a sharp rise in the top temperature. This was accompanied by the separation of an oily emulsion, which, in Runs 1 and 2, continued to come over until the temperature reached 100° C. A portion of this emulsion was extracted with ether over anhydrous sodium sulphate, and the ether layer separated and distilled. Two, small, constant temperature fractions were obtained, one boiling at 92° C. and the other at 127° C. The first fraction, which separated into two layers, appeared to be the *n*-butanol–water azeotrope (boiling point 92.25° C. (6)) or the isoamyl–alcohol–water azeotrope (boiling point, 95.15° C. (6)). A 3,5-dinitrobenzoate derivative was prepared from the upper layer, and repeated crystallization gave a constant melting point of 52.5° C. The 3,5-dinitrobenzoate of *n*-butanol melts at 64° C. (12) and that of isoamyl alcohol at 62° C. (12). Therefore the product obtained was neither of these. Further identification work on this unknown could not be continued because of the small amount available.

The fraction boiling at 127° C. had a refractive index of 1.4079 at 25° C. The boiling point and refractive index indicated that the material might be either 2-methylbutanol-1 or 2-methylpentanol-3. It was found to form a 3,5-dinitrobenzoate, which, on repeated recrystallization, had a constant melting point of 82° C. The melting point of the 3,5-dinitrobenzoate of 2-methylbutanol-1 is 62° C. (12) and that of 2-methylpentanol-3 is 85° C. (3). A sample of the latter was not available so it was not possible to do a mixed melting point.

The boiling point curve for Run 3 gave no indication of the presence of acetone. In order to prove its presence in this high wine more of the low boiling fractions were required. Four kilograms of the original high wine was distilled in five 800 gm. batches in a glass column packed with $\frac{1}{8}$ in. glass helices. Each batch was distilled with a reflux ratio of approximately 2 and the distillation was continued until 125 ml. had been collected. The 557 gm. of combined distillate was refractionated in a glass column 13 mm. in diameter, packed with 60 cm. of $\frac{1}{8}$ in. glass helices. The method of operation of both these columns was similar to that described above, in that the vapours were totally condensed, a portion of the condensate removed, and the rest returned to the column as reflux. The reflux ratio could not be measured but the rate of product removal could be carefully controlled. This last feature is an advantage over a cold finger condenser where the rate of take-off is controlled by adjusting the heat input to the still and water rate through the condenser. Under total reflux an initial temperature of 49° C. was obtained, while that obtained in the Stedman column was 57° C. The distillation curve is shown in Fig. 5. A constant temperature of 56.3° C. was observed in the range 0.8 to 3.0% distilled; this material was 0.31% of the high wine. The 2,4-dinitrophenylhydrazine derivative of this fraction was prepared and found to have a melting point of 126° C., which did not change when the product was mixed with an authentic sample of acetone 2,4-dinitrophenylhydrazine.

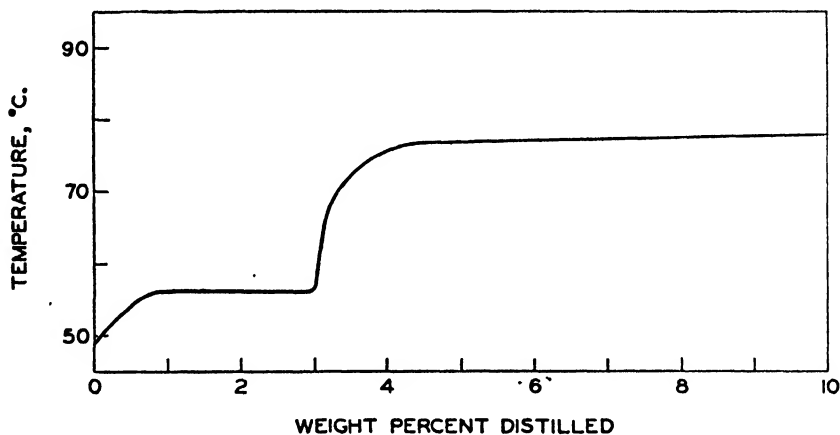


FIG. 5. Distillation curve of aldehyde fraction.

One sample of Run 3 boiling at 92° to 98.2° C. separated into two layers on condensation. The fusel oil layer was 0.015% of the high wine. This quantity was insufficient for further identification other than by solubility tests, which showed that it consisted of alcohols, aldehydes, and ketones with fewer than nine carbon atoms (12).

The presence of diacetyl in Run 3 was indicated by a characteristic yellow colour but was not proved by preparation of derivatives. In this run, how-

ever, only fractions boiling below 78.2° C. were coloured; this indicated that the diacetyl had been removed in the early part of the rectification. This difference is believed due to the higher reflux ratios used and to a lower initial content.

Discussion

The results presented above show that the high wine from a whole wheat mash fermented by *A. polymyxa* can be fractionated by distillation to give a low boiling fraction high in aldehydes, ketones, acids, and esters, a high boiling fusel oil fraction, and in between a fraction consisting of relatively pure ethanol at approximately the azeotropic composition. Thus, except for the presence of diacetyl this high wine has the same general composition as that obtained from a yeast fermentation, and can be purified by similar methods.

The average values of acid and ester in high grade industrial ethanol are 0.02 to 0.08 and 0.12 to 0.38 m.e. per 100 gm. of 95% ethanol.* From Table II it is seen that the acid content of Run 2 and ester content of Runs 1 and 2 are above these values. Although no information on the fusel oil content of industrial ethanol was available it is believed that the product of Run 2 is also high in fusel oil. The aldehyde contents of all materials listed in Table II were satisfactory; hence the product of Run 3 was satisfactory in all respects. It is of interest to know the percentages of total ethanol contained in these fractions. From the data of Tables I and II, and, taking the ethanol content of the rectified ethanol as 93%, a simple calculation shows that the percentages of total ethanol recovered were: 82.7% in Run 1, 77.9% in Run 2, and 84.5% in Run 3. Most of the remaining ethanol could, of course, have been recovered by further rectification of the other fractions.

The ethanol products of Runs 1 and 2 were also unsatisfactory because of a yellow colour, caused by diacetyl. It is thus apparent that further purification of these two products was necessary. It was proved that diacetyl can be removed by distillation over sodium hydroxide. The results of Run 3 indicate also that it can be removed by more efficient rectification. Although diacetyl boils at 88° C. it is removable in the aldehyde fraction because of its solubility properties. Either the addition of sodium hydroxide or more complete rectification would also reduce the acid and ester contents of the ethanol fractions. The choice of method to be used would depend on economic considerations. If the high wine were purified batchwise, sodium hydroxide could be added to the high wine charge, or to the first ethanol product. The latter procedure would require a redistillation, but would decrease the hydroxide consumption since less acid and ester would be present. In a continuous process, hydroxide could be added to the high wine feed or to the column from which the final product is removed.

* By courtesy of Joseph E. Seagram and Sons, Inc.

Acknowledgments

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PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

XIV. BUTANEDIOL ANALYSES FOR PROCESS CONTROL¹

By J. D. LESLIE² AND A. CASTAGNE³

Abstract

The chemical determination of 2,3-butanediol in fermented whole wheat mash and in certain materials from the recovery process has been investigated from the point of view of process control. The method of analysis involved extraction with *n*-butanol followed by estimation of the butanediol with periodate oxidation. Errors in both parts of the method were estimated and correction factors determined for the various types of materials. The factors recommended are: 1.039 for pure diol solutions and 1.067 for all other materials. Formulae are given for converting analytical results on beer to a basis of whole mash or beer-still slops. These calculations require a knowledge of the insoluble solids contents of the mash or the slops concerned.

Introduction

The method of analysis for 2,3-butanediol discussed in this paper has been found, in general, to be the most suitable in routine work. It consists of two steps: extraction of diol with *n*-butanol and determination of diol in the extract by periodate oxidation. The details of this method were developed by the Department of Biochemistry, University of Wisconsin.* It was applied there mainly to analysis of laboratory cultures, and an extraction coefficient of 96% was reported. During the early work in these laboratories the extraction coefficient was given little attention, since nearly all fermentations were on whole wheat mash of equal concentration and the results were chiefly of comparative value. It was only after study of the recovery phases of the pilot plant process had begun that analysis of materials other than fermented mash were required. Such materials included crude diol and pure diol concentrates, evaporator syrups, and distillates.

The process referred to for producing 2,3-butanediol was developed at the National Research Laboratories and has been described in various reports and papers (2, 3). So far the work has been confined to whole wheat mash fermented by *Aerobacillus polymyxa*.

The main purposes of the present work were to examine critically the analytical procedures for diol determination, to supply correction factors for the principal materials in the process, and to give the necessary calculation for applying the analytical results to process control.

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* Unpublished method.

Methods and Materials

Butanediol Analysis

Five millilitres of sample and 10 ml. of *n*-butanol, reagent quality, are pipetted into a 15 by 150 mm. test-tube; 6 gm. of anhydrous potassium carbonate is added and the mixture is shaken immediately for at least 15 sec. After standing for 2 to 3 min. (to permit complete hydration of the carbonate), the mixture is shaken for a further 30 sec. to ensure complete extraction of the diol. The carbonate should be almost completely dissolved. The tube and contents are centrifuged long enough to give two clearly separated layers, and then 5 ml. of the butanol layer is carefully pipetted into a 100 ml. volumetric flask, dissolved in distilled water and made up to volume. To a 5 ml. aliquot of diluted extract, in a 25 by 200 mm. test-tube, is added 1.0 ml. of 1.0 *N* sulphuric acid and 5 ml. of 0.01 *M* potassium periodate. After mixing, the tube is loosely capped with a glass stopper and heated in boiling water for exactly 10 min. After cooling, 5 ml. of 0.5 *M* disodium hydrogen phosphate is added, the mixture is shaken and 1 ml. of potassium iodide solution (30 gm. per 100 ml.) is added. The liberated iodine is titrated immediately with 0.005 *N* sodium thiosulphate. To correct for oxidizable substances in the butanol a blank determination on 5 ml. of distilled water is made in exactly the same way. The difference between the volumes of thiosulphate required to titrate the blank and the sample is used in calculating the diol content. One millilitre of 0.005 *N* thiosulphate is equivalent to 0.225 mgm. of 2,3-butanediol, and since the original 5 ml. sample is diluted 40 times in the procedure, the diol, in grams per 100 ml. of sample, is given by: ml. of thiosulphate $\times 40 \times 20 \times \frac{0.225}{1000}$ = ml. of thiosulphate $\times 0.180$. This cal-

culatation assumes 100% extraction, and the answer must be multiplied by an empirical correction factor as described in the next section.

Acetoin interferes quantitatively, 88 mgm. of acetoin appearing as 90 mgm. of diol. Ethanol up to 5% and glucose up to 20% do not interfere.

Materials and General Procedure

The materials in which diol estimations are required fall into four classes: (a) fermented mashes, beer-still slops, filtrates and beers, (b) crude diols from rectification and condensates from high pressure stripping, (c) evaporator syrups, (d) 'pure' diol concentrates. Some of these materials can be analysed directly as they come from the process, the rest require preliminary treatments as follows.

Mashes and beer-still slops are filtered through No. 1 Whatman paper; filtrates and beers are handled similarly if suspended materials are present. The prepared samples from these materials are essentially the liquid phase of fermented mash and are all called 'beer'. No dilution is made prior to analysis.

Evaporator syrups contain 10 to 20% diol and 30 to 60% solids. Five-gram samples are weighed out, dissolved in warm water, and made up to

100 ml. each. Occasionally a slight residue of fine particles is noticeable, but thorough shaking ensures that a true aliquot of the 100 ml. is taken for analysis.

Crude diol has a concentration of 50 to 70% and requires 20 to 40 times dilution; stripped condensates require two to five times dilution. 'Pure' diol concentrates are treated similarly, the dilution factor varying with the concentration.

These procedures require adjustment of the diol concentration below 3.5% and the soluble solids below 3.0% in the 'prepared' samples.

In determining correction factors for each of the above types of material, the general method was as follows. Known amounts of pure diol were added to a series of known quantities of each type and the series of samples were prepared for analysis as described above. All samples were made up to 100 ml. in volumetric flasks. Materials listed under (a) and (b) required more dilution during sample preparation than is indicated above, to allow for the added diol. Fifty grams of beer and 1.5 to 2.0 gm. of crude diol were taken per each 100 ml. sample. It is worth noting that all materials were weighed, whereas in routine analysis all but evaporator syrups are measured by volume. The latter procedure is faster and is accurate enough for practical purposes.

Pure diol prepared in the laboratory was used in making up the samples and served also as control. After repeated vacuum rectification over calcium oxide it had the following properties: $n_D^{25} = 1.4305$, b.p. = 178.5° C. at 760 mm. pressure, and $(\alpha)_D^{25} = -12.75^\circ$. Subsequent tests showed this diol to contain 0.45% moisture and 0.94% acetate radical. On the assumption that the acetate was completely hydrolysed when the samples were made up, the purity of the diol was taken as 98.88%.

Each series of prepared samples was analysed by making triplicate extractions followed by single diol determinations on each extract. 'Diol recovered' was plotted against 'diol added' and for each series a linear regression line was fitted to the points by statistical methods. The regression coefficient of each line gave a measure of the required correction factor, and the intercept on the 'diol added' axis gave the diol present in the original material, subject to any systematic error in the analysis.

To make the analytical results applicable to process control, it is necessary to determine total and soluble solids in filtrates and mashes. Weighed samples were air-dried at 100° C. for 48 hr., after which the residual diol in the dried samples was extracted with warm water and determined in the usual manner.

Experimental

Factors Affecting Diol Determination

Because of inconsistent results from the routine plant analyses some parts of the analytical procedure were investigated for errors before the main work was undertaken.

(1) The amount of anhydrous potassium carbonate added to a 5 ml. sample in conjunction with extraction by *n*-butanol was varied from 5.5 to 7.0 gm.

No significant effect was observed but 6.0 gm. is recommended since this is only slightly in excess of the amount required to saturate the sample. To avoid lumping it has been found advisable to shake the samples immediately upon addition of the carbonate.

(2) In blank determinations of diol the time of heating at 100° C. after addition of potassium periodate was varied from 5 to 20 min. The results for two experiments, shown in Table I, indicate a decreasing titre with increasing time of heating. The difference between corresponding values in the two experiments resulted from slight variations in the strengths of the reagents. A suggested explanation for the titre drop is that periodate undergoes slow decomposition during heating. It is therefore essential that the time of heating for blanks and unknowns be kept the same.

TABLE I
EFFECT OF HEATING TIME ON BLANK TITRATION
IN 2,3-BUTANEDIOL ANALYSIS

Experiment 1		Experiment 2	
Time, min.	Na ₂ S ₂ O ₈ titre, ml.	Time, min.	Na ₂ S ₂ O ₈ titre, ml.
7	19.10	5	19.56
15	18.86	10	19.45
20	18.68	15	19.30
		20	19.06

(3) After removal from boiling water, samples were allowed to stand for various periods in cold water before addition of buffer. No significant effect was observed.

(4) The time elapsing between addition of buffer and addition of potassium iodide was varied from 10 min. to two hours. Again no significant effect could be detected.

To obtain an estimate of the experimental error in the periodate estimation of diol, duplicate analyses were made on each of two sets of butanol extracts. The means and differences between the duplicates of these results are given in Table II. It is clear that the error in question is negligible.

Effect of Concentration on Direct Determination of Diol

To check the accuracy of the periodate estimation over the usual concentration range, aqueous solutions were prepared containing 0.5 to 3.6% diol. Extraction with *n*-butanol was omitted and, after equivalent dilution, duplicate determinations were made on each sample. To check the results obtained, the experiment was repeated with a new sample of diol of 99.17% purity. Table III gives the data of these tests; the differences between duplicate determinations are recorded as well as the mean recoveries. The results show that high recoveries are obtained with periodate oxidation in dilute solutions. This effect has been noted by previous workers (1) and is not

TABLE II
EXPERIMENTAL ERROR IN DIRECT DETERMINATION OF
2,3-BUTANEDIOL IN BUTANOL EXTRACTS

Pure diol series		Beer series	
Mean diol recovered, gm./100 ml.	Difference between duplicates, gm./100 ml.	Mean diol recovered, gm./100 ml.	Difference between duplicates, gm./100 ml.
1.003	0.000	1.092	0.005
1.233	0.000	1.341	0.000
1.450	0.002	1.503	0.000
1.917	0.000	2.013	0.005
2.313	0.000	2.473	0.004
2.813	0.009	2.889	0.000
Mean diff. = 0.0018		Mean diff. = 0.0023	

TABLE III
EFFECT OF CONCENTRATION ON DIRECT DETERMINATION OF 2,3-BUTANEDIOL

Diol of 98.88% purity			Diol of 99.17% purity		
Conc., gm./100 ml.	Mean recovery, %	Difference between duplicates, %	Conc., gm./100 ml.	Mean recovery, %	Difference between duplicates, %
0.5204	101.1	1.3	0.4972	101.1	2.5
1.0408	99.9	0.6	0.9942	101.6	1.6
1.5612	99.6	0.2	1.4916	100.0	0.3
2.0816	98.8	0.5	1.9888	99.5	0.7
2.6020	98.0	0.0	2.4860	99.1	0.2
3.1224	97.8	0.1	2.9832	98.7	0.3
3.6428	97.5	0.0	3.4804	98.7	0.0

Note.—At 0.5 gm./ml. concentration, difference of 0.03 in titre = difference of 1.3% in recovery.

easily explained. The present work suggests that in dilute solutions there is slight decomposition of the residual periodate and this appears as additional diol in the final answer.

Determination of Correction Factors

To estimate the correction factors for the various types of material the following experiments were carried out as described above: one with pure diol solutions, three with beers, three with syrups, and two with crude diol products. Fig. 1 shows one series of each type of material. The 'diol recovered' was calculated from the means of the several extractions. The final results are summarized in Table IV.

Analysis of variance showed that, among the regression coefficients, only the value for pure diol was significantly different from the others. Hence, one value, 0.9264, is recommended for all materials other than pure diol solutions;

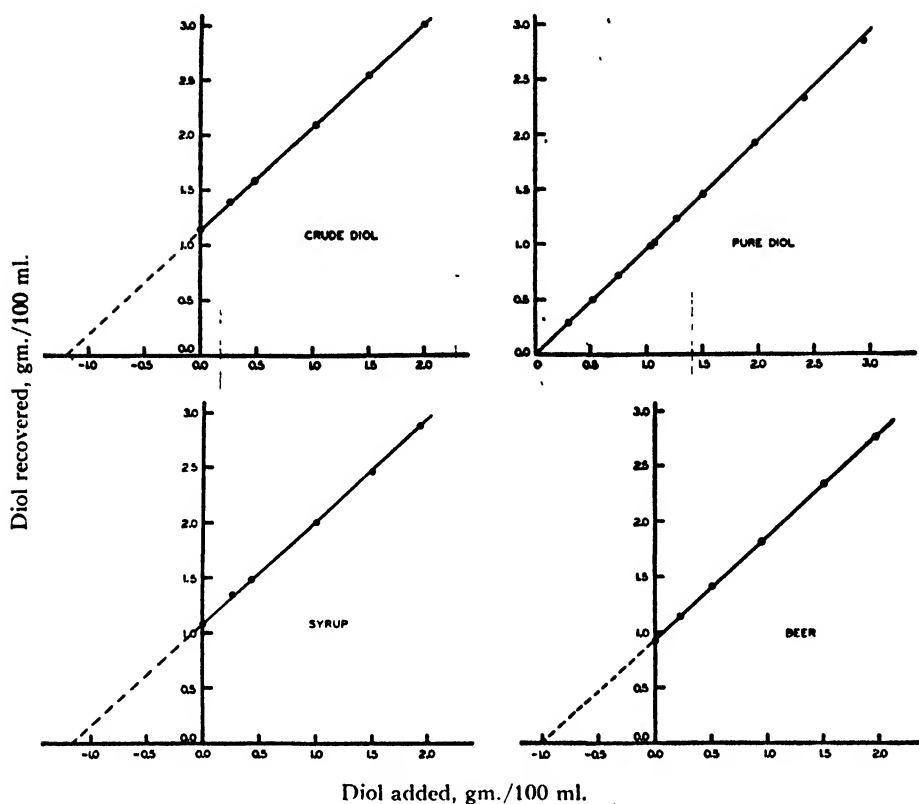


FIG. 1. Recovery of 2,3-butanediol in analysis of various process materials.

TABLE IV
CORRECTION FACTORS FOR 2,3-BUTANEDIOL ANALYSIS IN
VARIOUS PROCESS MATERIALS

Series	b	S_b	S_e
Beer 1	0.9246	0.0039	0.0101
Beer 2	0.9252	0.0067	
Beer 3	0.9244	0.0068	
Syrup 1	0.9198	0.0085	0.0236
Syrup 2	0.9385	0.0066	
Syrup 3	0.9094	0.0222	
Crude diol 1	0.9303	0.0041	0.0149
Crude diol 2	0.9401	0.0116	
Average	0.9264	0.0088	
Pure diol	0.9514	0.0015	0.0038

b = Regression coefficient, S_b = standard error of b , S_e = standard error of estimate.

for the latter the value 0.9514 should be used. It is believed, however, that repetition with a greater number of series might show a significantly different regression coefficient for crude diol products.

For ease of computation in routine work it is desirable to use the formula: diol present = diol by analysis \times correction factor. The best estimates of these correction factors are obtained by dividing the diol purity (in this case 0.9888) by the corresponding regression coefficient, and are therefore: 1.039 for pure diol solutions, and 1.067 for all other materials. From the standard errors of estimate, given in Table IV, it appears that the factors are most reliable with pure diol solutions and least reliable with syrups.

It is believed that these results can be applied to the *Aerobacter aerogenes* fermentation process also, because the products are similar.

Insoluble Solids in Mashs and Beer-still Slops

To calculate diol concentrations on the basis of fermented mash the amount of insoluble solids present must be known. The same absolute amount of insolubles may be used in connection with the corresponding beer-still slops providing no appreciable change in the distribution of soluble and insoluble solids takes place during passage through the beer still.

This problem was investigated as follows. Total solids were determined in a sample of fermented mash and soluble solids in a filtrate from this mash. A portion of the mash was then boiled at atmospheric pressure for 15 min. (= approximate retention time in beer still), under a reflux condenser to prevent loss of material from the boiling sample. The boiled mash was then cooled and filtered, and soluble solids were again determined in the filtrate. No pH adjustment was made in any of the samples. All determinations were done in duplicate, and the results for two experiments are given in Table V. These results indicate that soluble solids in the mash were slightly increased during the heating operation. In most calculations, however, the effect can be ignored since, as will be shown in the next section, it enters only into the factor, $100 - \text{percentage of insoluble solids}$.

Application of Results

It has been stated already that calculation of yields and material balances on diol-containing materials of widely varying characteristics necessitates careful consideration of the basis of calculation. This is especially true when large volumes with low diol contents are involved. No difficulty is encountered in dealing with syrups, crude diol products, pure diol concentrates, beers, and filtrates since these materials are essentially one phase, i.e., no insoluble solids are present. On the other hand, fermented mashs and beer-still slops are two-phase materials and the diol (and ethanol) concentrations on a liquid basis must be converted to a basis of whole mash (or whole slops).

The calculation is simple but requires knowledge of the insoluble solids. As described above, this quantity is best found by determining the total

TABLE V

EFFECT OF 15 MIN. BOILING ON DISTRIBUTION OF SOLIDS IN WHOLE
WHEAT MASH FERMENTED BY *A. polymyxa*

	Experiment 1		Experiment 2	
	Ferm. 1	Ferm. 2	Ferm. 1	Ferm. 2
Mash conc., %	13.1	14.0	14.8	14.7
Final diol, %	2.15	2.33	3.26	2.50
Final ethanol, %	1.33	1.47	0.50	1.32
	Mean diff.	Mean diff.	Mean diff.	Mean diff.
Total solids in mash before boiling, %	6.50 0.04	6.51 0.05	5.47 0.01	5.97 0.01
Soluble solids in filtrate before boiling, %	3.09 0.06	3.33 0.02	3.06 0.22	3.26 0.20
Soluble solids in filtrate after boiling, %	3.22 0.05	3.61 0.06	3.03 0.17	3.46 0.07
Av. diff.	0.05	0.04	0.13	0.09

solids in the mash and the soluble solids in the filtrate. The insoluble solids, P_i , as per cent of the mash*, are then given by the formula.

$$P_i = \frac{100 (P_t - P_s)}{100 - P_s},$$

where P_t = per cent total solids in mash,
and P_s = per cent soluble solids in filtrate.

The per cent diol, c , in whole mash, can then be calculated from the formula

$$c = \frac{c^1 (100 - P_i)}{s_1 100} = \frac{c^1 (100 - P_i)}{s_1 (100 - P_s)},$$

where s_1 = specific gravity of filtrate,

and c^1 = diol concentration in grams per 100 ml. of filtrate. Use of the second form of this equation eliminates a separate calculation of P_i .

If V = total volume of mash in Imperial gallons, and s_2 = specific gravity of mash, then

$$\text{Total lb. diol} = \frac{Vs_2 c^1 (10)}{s_1 (100)} \frac{(100 - P_s)}{(100)}.$$

The same formulae apply to the calculation of ethanol if c^1 is taken as grams of ethanol per 100 ml. of filtrate.

For a given mash concentration and assuming normal fermentations, i.e., 90% completion or better and no contamination, s_1 and s_2 are only slightly greater than unity and their ratio varies but little from one mash to the next.

* All percentages are by weight.

For 15% whole wheat mash s_1 may be taken as 1.01 and s_2 as 1.02. If, for the same conditions, the limits on P_i are taken as 2.5 to 3.5% then the factor $\frac{s_2}{s_1} \frac{(100 - P_i)}{100}$ will have limits 0.985 to 0.975. For approximate calculations, the insoluble solids may be taken as 3.0% and then:—

$$\text{Total lb., diol} = \frac{V (1.02)}{(1.01)} c^1 \frac{(10) (0.970)}{100} = 0.0980 V c^1$$

It is clear that for these conditions the insoluble solids may be considered to be unchanged in passing through the beer still, although their concentration may be altered if dilution or concentration of the mash takes place.

Acknowledgments

The authors wish to thank Dr. G. A. Adams, of the Division of Applied Biology, for his constantly helpful advice and criticism throughout this work, and Mr. E. L. Tollefson for checking the several points in the analytical method. Thanks are due also to Dr. J. W. Hopkins of the Divisional Staff, for assistance with the statistical analysis.

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PRODUCTION AND PROPERTIES OF 2,3-BUTANEDIOL

XV. THE OCCURRENCE OF ACETONE AS A PRODUCT OF THE *AEROBACILLUS POLYMYXA* FERMENTATION

BY DYSON ROSE²

Abstract

Considerable quantities of acetone were produced by certain strains of *Aerobacillus polymyxa* growing anaerobically in a dextrose medium. The presence of acetone was confirmed by formation of the 2,4-dinitrophenylhydrazine derivative. Carbon balances were obtained for both non-acetone-producing and acetone-producing strains of the organism; considerable quantities of several organic acids were also formed. Increased efficiency of nitrogen-aeration resulted in a marked lowering of the butanediol : ethanol ratio and probably is conducive to an increased yield of acetone.

Introduction

Donker (4) in 1926 presented evidence that the fermentation of dextrose by the organism *Aerobacillus polymyxa* led to the production of significant quantities of acetone, but earlier work in these laboratories did not support this conclusion. So far as we know, other workers have not reported the production of acetone by the organism. However, during more recent work with various strains of *A. polymyxa*, it was found that satisfactory carbon recoveries could not be obtained in all experiments if analyses were made for only the expected products. A consideration of the data obtained indicated that the substance overlooked might have been acetone, and the possible presence of this substance was therefore investigated.

Materials and Methods

The general methods and procedure of conducting the fermentations were the same as those previously described (3) except that Kluyver flasks (6) were used to provide more efficient nitrogen-aeration.

The analytical methods were largely those used by Neish (8), although some modifications were made during the course of this work. Malic acid was determined by the method of Pucher *et al.* (9), acetone by the method of Goodwin (5). All analyses were made at the close of a 96-hr. fermentation period.

Experimental Results

Various strains of *A. polymyxa* were studied but data for only two of these are presented. N.R.C. strain C2(3) was selected as representing the general type because it consistently gave 2,3-butanediol and ethanol yields approximating those commonly found in the fermentation of grain mashes (7). On

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the other hand, strain C25 under the same conditions produced less butanediol and more ethanol, and satisfactory carbon recoveries could not be obtained by the routine analytical procedures. Although no truly intermediate strains were found among those studied, considerable variation in both types of fermentation did occur.

Fermentation Products of Strains C2(3) and C25

Yields of the various fermentation products with strains C2(3) and C25, expressed as millimoles per 100 millimoles of dextrose fermented, are shown in Table I. The carbon recoveries of 97.2 and 98.3% are considered satisfactory as no attempt was made to correct for the amount of substrate assimilated by the growing culture. The ratios of the calculated: observed carbon dioxide and hydrogen yields, and the oxidation-reduction indices, are also satisfactory.

TABLE I
FERMENTATION PRODUCTS OF TWO STRAINS OF
A. polymyxa
Millimoles per 100 millimoles of dextrose dissimilated

Product	C2(3)	C25
Butanediol	45.5	40.6
Acetoin	2.8	0.2
Ethanol	76.7	83.2
Formic acid	1.2	2.4
Acetic acid	14.0	6.3
Lactic acid	3.2	2.9
Succinic acid	1.8	1.6
Malic acid	0.5	0.2
Carbon dioxide	188.3	200.7
Hydrogen	90.1	97.9
Acetone	Tr. (?)	9.5
Dextrose fermented	5077	5066
Carbon recovery, %	97.2	98.3
CO ₂ , calc./obs.	1.013	1.00
H ₂ , calc./obs.	0.998	1.001
O/R index	0.987	1.001
Acetoin + diol (by weight)	1.23	0.96
Ethanol		

These data differ from those of Adams and Stanier (3) in that formic, lactic, succinic, and malic acids are present in the fermented medium. The method used for estimating formic acid in the present work was more delicate than theirs, and it is not impossible that traces of formic acid were present in their medium even though high carbon recoveries were obtained. Succinic acid was obtained in all the present experiments so that strain differences would not appear to be an adequate explanation for its absence in the experiments recorded by Adams and Stanier. Wood and Werkman (12) have reported that various factors, especially the presence or absence of phosphate in the medium, affect the production of succinic acid by *Propionobacterium*, and Neish (8) has shown that differences in the culture media greatly affect

the yield of acids by *Bacillus subtilis*. No significant differences between our medium and that used by Adams and Stanier could be found and consistent changes in the amount of succinic acid formed could not be induced by minor changes in the medium. It seems probable that the formation of lactic and succinic acids is a function of the particular medium and conditions used.

In the absence of succinic acid, no tests for malic acid were made by Adams and Stanier. It is probable that the yeast extract rather than the dextrose was the source of malic acid; the amounts found were frequently less than those shown in Table I.

The acetone production shown for Strain C25 in Table I was calculated on the assumption that one mole of acetone and three moles of carbon dioxide are produced per mole of dextrose utilized, and that the ratio of calculated to observed carbon dioxide must be 1.0. If acetone is not included in the balance, the carbon recovery amounts to only 93.5%, the ratios of the calculated to observed carbon dioxide and hydrogen are 0.86 and 0.61 respectively, and the oxidation-reduction index is 1.05. Obviously such values indicate that some product has been overlooked, and the improvement that results in all these values when acetone is included, computed on the basis of the carbon dioxide found in excess of that calculated, provides strong evidence that acetone is the missing product.

Analyses for acetone were later conducted on a medium fermented by Strain C25 under similar conditions and gave clear evidence that a considerable amount of this product was being produced. Positive identification of the acetone produced in these fermentations was made by means of the melting point and mixed melting point of the 2,4-dinitrophenylhydrazine derivative.

On the other hand, the data in Table I indicate that acetone is not produced in sufficient quantity to affect the carbon balance for the C2(3) fermentation, and subsequent tests with cultures in 200-ml. flasks tended to support this conclusion, although slight positive results were obtained when analyses for acetone were made by the iodoform method. These results indicate an acetone content of about 0.01% in the fermented medium, and were greater than could be explained on the basis of the interference due to ethanol. It therefore appears probable that even with this strain of the organism traces of acetone were formed under the conditions used in these fermentations.

While the work recorded in this paper was in progress, Tollefson *et al.* (11) found that acetone could be recovered by rectification of the low boiling fraction of the medium fermented by *A. polymyxa*. This observation does not prove that acetone is produced by *A. polymyxa*, owing to the possibility that contaminated fermentations were included, but it does provide confirmatory evidence.

The Effect of Nitrogen-aeration on the Fermentation

Although the usual ratio of butanediol to ethanol obtained in anaerobic fermentations with *A. polymyxa* is about 1.3 : 1 by weight (1), values of less than 1.0 : 1.0 have been consistently obtained in fermentations that produce

significant quantities of acetone. Adams and Stanier (3) suggested that differences in this ratio are due to variations among the strains of the organism, and our experience has tended to support the suggestion. It has been found, however, that differences in the rate and efficiency of nitrogen-aeration tend to augment the effect of strain differences. Table II presents data that serve to illustrate this effect, from six experiments conducted with Strain C25 under varying degrees of nitrogen-aeration.

In some of these experiments (Method I) approximately equal amounts of nitrogen were admitted in either coarse or fine bubbles, while in others (Method II) varying amounts of nitrogen were admitted in fine bubbles. The rates of nitrogen-aeration for the latter are recorded together with the acetone yields in Table III.

The data in Table II show that increasing the degree of nitrogen-aeration by either of these methods results in a decrease in the butanediol: ethanol ratio. The differences between the means for Method I are statistically significant, indicating that coarse bubbles result in a higher butanediol: ethanol ratio as compared with fine bubbles. No significant difference was shown between the effects of slow and fast aeration.

TABLE II

THE EFFECT OF THE RATE OF NITROGEN-AERATION ON THE BUTANEDIOL : ETHANOL RATIO

Method I

Exp. no.	Coarse bubbles			Fine bubbles		
	Diol	Ethanol	Ratio	Diol	Ethanol	Ratio
1	0.302	0.278	1.09	0.230	0.264	0.87
2	.359	.254	1.41	.249	.259	.96
3	.262	.268	0.98	.141	.251	.56
Average			$1.16 \pm .18$			$0.80 \pm .17$

Method II

Exp. no.	Slow aeration			Fast aeration		
	Diol	Ethanol	Ratio	Diol	Ethanol	Ratio
1	0.165	0.237	0.70	0.122	0.244	0.50
2	.144	.231	.62	.120	.217	.55
3	.143	.208	.69	.122	.195	.63
Average			$0.67 \pm .04$			$0.56 \pm .05$

Method I:—Kluyver flasks, coarse bubbles of nitrogen admitted through a glass tube inserted through the top of the flask; fine bubbles of nitrogen admitted through the sintered glass disk.

Method II:—Kluyver flasks, nitrogen admitted through the sintered glass disk at varying rates. Cf. Table III.

The production of acetone by Strain C25 in some of these experiments is shown in Table III. The average amount of acetone found is approximately double that calculated for Table I, in which the data were obtained from a medium that was nitrogen-aerated at a rate of about 60 to 70 cc. per litre per min. The data in Table III also indicate that the yield of acetone tends to increase with increasing rates of nitrogen-aeration, but since good control of the rates of gas flow was not obtained these results are not conclusive. Since

TABLE III

THE EFFECT OF THE RATE OF NITROGEN-AERATION ON PRODUCTION OF ACETONE BY *A. polymyxa* STRAIN C25

Approx. rate of nitrogen-aeration, cc./litre/min.	Acetone, % of medium		Total
	Retained in medium	Recovered from trap*	
20	0.036	0.001	0.037
100	.045	.004	.049
50	.057	.016	.073
150	.033	.045	.078
75	.028	.035	.063
300	.024	.067	.091

*A sodium bisulphite trap was used to collect the acetone swept out by the nitrogen stream. The acetone thus caught was later released by the addition of alkali and recovered for analysis by distillation.

increased aeration tends to remove more acetone from the medium, an attempt was made to decrease its production by the addition of acetone at the time of inoculation, but the subsequent evaporation of the added acetone obscured the results. Indications were, however, that the added acetone had no effect on the fermentation, and it seems doubtful whether the apparent effect of aeration on the acetone production can be directly due to the sweeping out of this product as it is formed.

Discussion and Conclusions

The fact that certain strains of *A. polymyxa* are capable of producing a considerable quantity of acetone is adequately proved by the data presented. The amounts of acetone obtained with Strain C25 under our conditions greatly exceed those recorded by Donker (4), and are sufficient to affect the commercial production of butanediol by means of this fermentation. However, since strains used in commercial plants would be selected for a favourable butanediol : ethanol ratio there appears to be little likelihood of acetone-producing strains being used. The low butanediol : ethanol ratio obtained in all cases where acetone was formed indicates that this product arises at the expense of the diol while the ethanol production remains relatively constant or even increases.

The significant effects exerted by the rate and efficiency of nitrogen-aeration on the butanediol : ethanol ratio, and probably on the acetone production, are of considerable interest. The most probable explanation is that some product of the fermentation reactions is removed from the medium by the gas stream. The data available do not favour the conclusion that the product thus being removed is the acetone itself. Adams (1) and Adams and Leslie (2) have shown that the removal of carbon dioxide from the fermenting medium has a marked effect on the fermentation. It is significant, however, that under conditions of excess aeration with nitrogen, in spite of the decreased diol production, no increase in ethanol or any other product whose precursor acts as an hydrogen acceptor is evident. This is in contrast to the strain differences noted in Table I and would seem to indicate that hydrogen is being rapidly swept from the medium instead of being used to reduce some intermediary product. The formation of acetone from pyruvate through acetic and aceto-acetic acids involves the removal of hydrogen as well as carbon dioxide, and the fate of this hydrogen is not known. It seems possible that the increased formation of acetone under conditions of efficient nitrogen-aeration may be at least partly due to the increased removal of gaseous hydrogen from the medium.

The ability of *Aerobacillus* strains to produce variants, and the need for constant selection if a parent strain is to be kept pure, is well known. It is worth noting, however, that the acetone-producing strain used in these experiments (C25) is a descendant of the organism used by Adams and Stanier (3). From time to time during the intervening two years this organism has been plated out and any variants differing morphologically from the original were eliminated. In spite of this repeated selection the fermentation induced by the strain now bears little resemblance to that of the parent culture, and it seems unlikely that this is entirely due either to differences in the medium used or to the increased efficiency of nitrogen-aeration. The conclusion that considerable physiological variation has occurred without noticeable variation in morphological form seems inescapable and the necessity for frequent testing of strains is thus distinctly emphasized.

Acknowledgment

The author wishes to express sincere thanks to the Department of Biochemistry, University of Toronto, for the facilities provided during part of this investigation.

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DRIED WHOLE EGG POWDER

XXI. PASTEURIZATION OF LIQUID EGG AND ITS EFFECT ON QUALITY OF THE POWDER¹

BY N. E. GIBBONS², C. O. FULTON³, AND MARGARET REID⁴

Abstract

Heating liquid egg to 60°C. reduced the viable bacterial count 85 to 95% but had less effect on the coliform organisms. Holding the liquid at 60°C. for 30 min. reduced the viable count 98 to 99%, and destroyed the coliforms, *Salmonella*, and *Staphylococci*. Quality tests indicated no difference between powders prepared from heated and unheated melange either originally or after storage. Tests with a laboratory flash pasteurizer indicated that a considerable reduction in the number of total viable and coliform organisms occurred when liquid egg was heated from 22° to 60°C., the total time in the pasteurizer being approximately one minute.

Introduction

The presence of organisms of the *Salmonella* group (4) and coagulase positive *Staphylococci** in dried egg powder introduced a public health hazard that at one time was viewed with some alarm. Previous studies indicated that in the interests of quality the temperature of drying should be as low as possible and the powder should be cooled as rapidly as possible (12). Both of these recommendations favoured the survival of bacteria during the drying process (3). The present studies were undertaken to determine the feasibility of destroying possible pathogens by heat treating liquid egg and to evaluate the effect of this treatment on the keeping quality of the powder.

Although the principle of heating liquid egg to facilitate drying was well known, little information was available on the heat treatment of liquid egg with the object of destroying bacteria. It has been stated that broken out duck eggs may be pasteurized by adding sodium citrate, heating to 65°C. for 20 min., and cooling rapidly (10). In a criticism of this method it was claimed that the heating period of 20 min. was not always sufficient to kill *Salmonella* (11). Recently some work on hens' egg melange has been done in the United States (5).†

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* Unpublished data.

† Since this paper was submitted, this work has begun to appear in more detail.

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Materials and Processing Methods

Preliminary tests indicated that fresh egg liquid could be heated to 52° and 59°C. (126° to 138°F.) for periods up to 12 hr., with a reduction in the bacterial content, but without affecting the fluorescence value of the powder prepared from it. Fresh melange could be heated to 66°C. (151°F.) and held 30 min. with no evidence of coagulation, provided it was stirred vigorously. There was some coagulation when the temperature was raised to 68°C. (154°F.). It was not possible to obtain these temperatures in the commercial trials with the equipment available as the temperature differentials necessary were so high that coagulation occurred on the heating coils; the highest practical temperature was 60°C. (140°F.).

Liquid egg was divided into two portions, one of which was inoculated with *Escherichia coli*, and the other with an organism resembling *Streptococcus faecalis*. Portions of each lot were heated in 5 to 10 min. to 55°, 57°, and 60°C. (131°, 135.5°, and 140°F.). After one-half, one, two, three, and four hours, samples were removed for bacteriological analysis and for drying. The samples for drying (100 ml.) were frozen immediately at -40°C. and dried in a vacuum over calcium chloride as soon as possible. The powders were stored at -40°C. until all had been prepared. Fluorescence and potassium chloride were then determined and the powder stored 21 days at 37°C. when the fluorescence was again measured.

Based on the results of these tests, further experiments were made with mixtures of *E. coli*, *E. freundii*, and *Aerobacter aerogenes*, of *Salmonella*, and of hemolytic *Staphylococci* and *Streptococci*.

For the first commercial trial, 2000 lb. of liquid egg prepared from frozen blocks was used. The drier and all tanks and lines had been cleaned some hours previously and filled with hypochlorite solutions and rinsed with hot water. The melange was heated to 60°C. in a cream forewarmer, consisting of an open uninsulated metal tank with a rotating helix through which hot water was circulated at 82°C. (180°F.). The mass was heated from 8° to 60°C. in 54 min. This time could probably have been shortened but the temperature at which the egg might coagulate on the coils was not known. After holding the liquid 30 min. at a temperature of 59° to 60°C., it was pumped into an insulated holding tank and fed as needed through a line filter into a small ballast tank near the drier. All of the liquid was dried 2.5 hr. after pasteurizing. The temperature of the melange and outlet air temperature of the drier are given in Fig. 1. Samples of powder were collected 10, 85, and 145 min. after drying began and immediately cooled. As soon as the heated egg was dried, the drying of unheated liquid from the same master batch was resumed and after 30 min. of operation a sample of powder was collected as a control. All samples were analysed bacteriologically and chemically at once and after storage for three weeks at 37°C.

In a second trial, five lots of 2000 lb. each were processed. The melange was prepared the day before, cooled, and held at 4°C. (39°F.) overnight. The

holding temperatures and times are given in Table III. Lot *B* was raised to temperature more rapidly than *A*. The control powder (*F*) was prepared from the same master batch of melange immediately after the others were dried. Throughout the trials the temperature of the water entering the coils

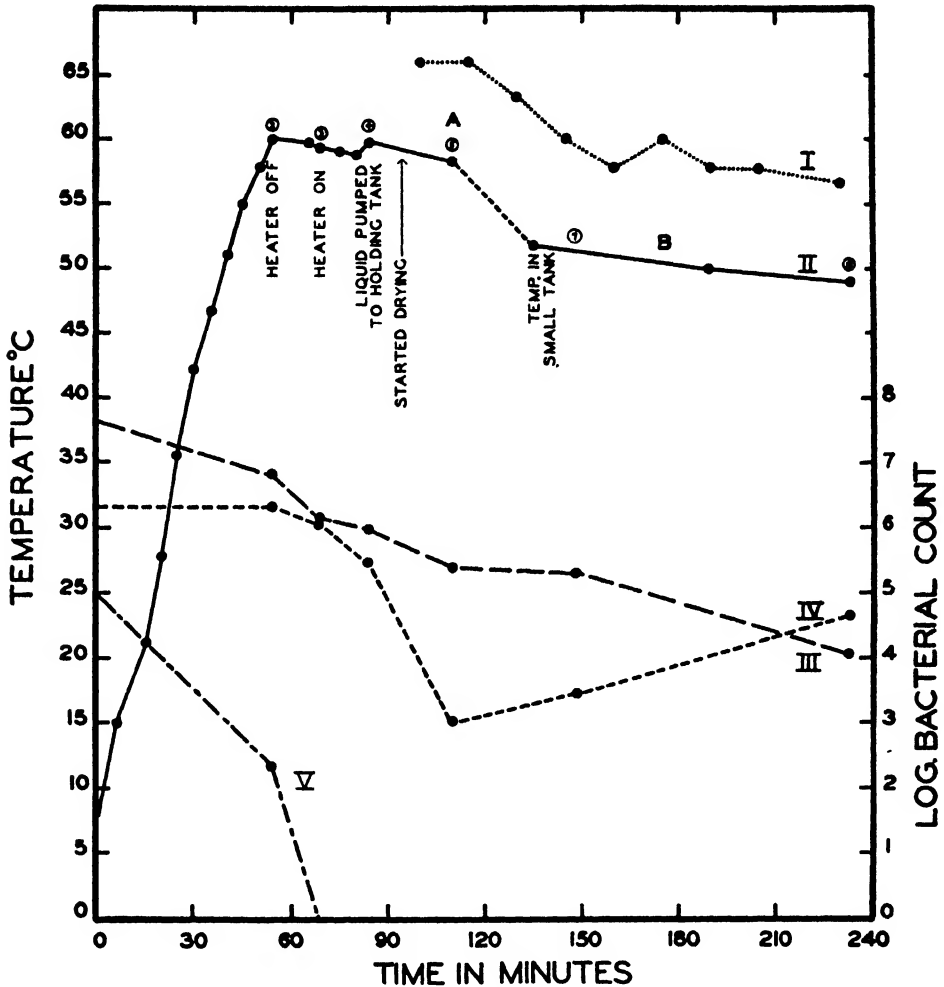


FIG. 1. Data regarding first commercial run. I—outlet air temperature of drier; II—temperature of liquid egg; III—viable bacterial count at 37°C.; IV—viable bacterial count at 55°C.; V—coliform content; 1, 2, etc.—samples of liquid egg taken; A, B—samples of powder taken.

was between 81° and 86°C. (178° to 187°F.). The air inlet temperature to the drier was 96°C.; the outlet temperature varied between 57° and 60°C. On leaving the drier the powder was cooled in a ramshorn with air at 5°C. Approximately 10 lb. from the first of each run was excluded from the test material. Samples for analysis were taken as the powder was being sifted into barrels.

Between lots, all lines and tanks, with the exception of the ballast tank, were washed with hot water and steam. The ballast tank was merely washed with hot water to allow continuous operation of the drier.

The powder was stored at 16°C. (60°F.) and packaged after five days. At that time 14 lb. of test material was removed from each lot by taking small amounts throughout the packaging operation. This was stored at -1.1°C. (30°F.) for six days, then sealed in cans and stored at 32.2°C. (90°F.) for periods up to four weeks, at 26 7°C. (80°F.) up to 12 weeks, and at 21 1° (70°F.) and 15.6°C. (60°F.) up to eight months. The remainder of the powder was shipped overseas in the usual 14-lb. packages.

Since there are several disadvantages to vat pasteurization, a small experimental unit was constructed to explore the possibility of flash pasteurizing the liquid egg immediately before it entered the drier. Three glass tubes 80 cm. long with inside diam. of 3 mm. were enclosed in a water jacket made of glass tubing (41 mm. I.D.).

Water was pumped through the jacket from a constant temperature bath by means of a centrifugal pump. The egg was forced through the tubes (total capacity approximately 17 ml.) at the rate of about 20 ml. per min. by a constant volume delivery pump. Some liquid egg pasteurized in this apparatus was pumped directly into a laboratory spray drier (14).

Analytical Methods

Viable bacterial counts were made on proteose-peptone-tryptone agar after incubating at 37°C. (98°F.) for three days. In the first commercial trial, duplicate plates were also incubated at 55°C. (131°F.). The presence of coliform organisms was detected by inoculating appropriate dilutions into five tubes of brilliant-green bile broth. Gas production was checked after 24 and 48 hr. incubation at 37°C. and the most probable number determined (1).

Powder quality was evaluated by fluorescence value (8), potassium chloride value (9), moisture (9), and palatability (8).

Results

Effect of Pasteurization on Bacterial Content

The results of several preliminary experiments are summarized in Table I. At 60°C. the viable count reached a minimum and all coliforms had been killed in 30 min. At 57 5°C. this was not accomplished for at least three hours. Mixtures of at least two strains each of *E. coli*, *E. freundii*, and *A. aerogenes* and of five types of *Salmonella* were destroyed in 25 min. at 60°C. Six strains of α -toxin producing, coagulase-positive *Staphylococci* were killed in 15 min. at 60°C. and four strains of Group A *Streptococci* were killed in 10 min. at 60°C., although 20 min. was taken to attain the desired temperature. It would seem therefore that 60°C. for 30 min. is the shortest time possible for vat pasteurization.

TABLE I
EFFECT OF HEATING LIQUID EGG ON THE BACTERIAL CONTENT

Organism added	Approx. time to attain pasteurization temp., min.	Pasteurization temp., °C.	Holding time at pasteurization temp., min.	Log. no. organisms per ml.			
				Initial		After heating	
				Viable	Coliform M.P.N.†	Viable	Coliform M.P.N.
<i>E. coli</i> *	8	60	15	7.57	6.25	4.00	0.30
			30			3.45	0
			60			3.34	0
	5	57.5	30	7.73	7.85	4.22	1.34
			60			3.63	1.23
			120			3.48	1.34
<i>E. coli</i> ** <i>E. freundii</i> <i>A. xyloxylophilus</i> <i>Salmonella</i> spp.**	5	55.0	240	7.85	6.25	3.30	.30
			30			5.74	3.30
			120			4.04	2.20
	4	60	240	7.13	7.13	3.59	2.26
			10			3.73	
			20			1.18	
<i>Staphylococci</i> ** α -toxin, coagulase + <i>Streptococci</i> ** Group A <i>S. faecalis</i> *	4	60	25	7.26	7.26	0	
			10			3.04	
			20			1.70	
	20	60	25	7.50	7.50	0	
			0			2.60	
			15			0	
<i>Streptococci</i> ** Group A <i>S. faecalis</i> *	20	60	10	6.90	6.90	0	
			30			3.45	
			60			3.41	
<i>S. faecalis</i> *	10	60	120	4.70	4.70	3.32	

†M.P.N.—Most probable number of organisms.

*Added to melange containing other organisms.

**Added to melange containing < 10 other organisms per ml.

The first commercial trial was based on these results. The pertinent data and the decrease in bacterial content are shown in Fig. 1.

The high initial viable count was attributed to thawing frozen melange with warm water and failing to cool it promptly. It served admirably for the purpose of the test. The viable count at 37°C. had decreased to about one-sixth of the original by the time the temperature of the liquid reached 60°C. and at the end of the 30 min. holding time there was a 98% reduction. The reduction in count continued in the liquid awaiting drying, and at the final sampling was 99.97%.

The number of coliform organisms decreased from 92,000 per ml. initially to 220 per ml. by the time the temperature of the liquid had reached 60°C., and none could be detected in 10-ml. amounts after 15 min. heating.

Organisms capable of growing on plates incubated at 55°C. were reduced to only 15% of the original number at the end of the pasteurizing period. The number continued to decrease for a short time afterwards, but as the temperature of the melange approached 55°C. began to increase again. Since none

of these organisms is pathogenic, they are of little significance. However, their behaviour serves to point out some of the dangers inherent in batch processes and to emphasize the fact that the liquid should be dried as rapidly as possible after heating.

The powder prepared from the pasteurized liquid contained only a small fraction of the number of viable bacteria present in that prepared from untreated egg (Table II).

TABLE II

QUALITY MEASUREMENTS AND BACTERIAL CONTENT OF POWDERS PRODUCED FROM PASTEURIZED AND UNPASTEURIZED LIQUID EGG (FIRST COMMERCIAL TRIAL)

	Moisture %	Fluorescence value		Palata- bility ratings	Potassium chloride value	Viable bacterial count, log. no. per gm.	
		Initial	After 3 weeks at 37°C.			37°C.	32°C.
Pasteurized*							
<i>G</i>	2.77	13.5	43.3	7.5	68.2	4.71	4.82
<i>H</i>	3.05	14.0	42.7	8.0	72.5	4.64	4.62
<i>I</i>	2.80	15.0	40.0	8.5	70.5	4.54	4.58
<i>J</i> Unpasteurized	4.16	14.0	46.4	8.5	73.1	6.88	6.93

* Collected 10, 85, and 145 min. respectively after drying began.

TABLE III

EXPERIMENTAL DATA ON SECOND COMMERCIAL PASTEURIZATION EXPERIMENT

Lot	Holding temp., °C.	Time to attain temperature, min.	Holding time, min.	Moisture content of powder, %
A	60	69	30	3.75
B	60	36	27	3.60
C	60	48	0	3.68
D	57	36	10	4.02
E	57	64	0	4.16
F (control)	—	—	—	3.77

In the second commercial trial the heating-up period was the most destructive to the bacterial population, but within the limits studied the time taken to reach temperature had little effect (Table IV). The time of holding had little effect on the final number of organisms. The temperature attained was most important in determining the viable count in the liquid.

The heating-up period (Table IV) was as destructive to the bacteria as the whole pasteurizing period in the previous experiment (Table II). In the

control powder (*F*) the percentage reduction due to drying alone was as great as that obtained by both pasteurizing and drying. Although such high percentage reductions due to drying alone have been reported (7), they are not usual (2, 3) and the extent of reduction probably depends on the particular flora of the melange being dried. The data for coliform organisms are unfortunately not complete; nevertheless, the indications are that at these temperatures a holding period is necessary to eliminate these organisms from the melange (Table IV). The high coliform content of the powder produced

TABLE IV

LOGARITHM OF NUMBER OF VIABLE ORGANISMS GROWING AT 37°C. AND OF MOST PROBABLE NUMBER OF COLIFORMS AND *E. coli* IN EGG MELANGE AND POWDER IN SECOND COMMERCIAL EXPERIMENT

Sample*	Log. viable count, 37°C.					
	A**	B	C	D	E	F
1	6.39	6.53	6.60	6.28	6.83	—
2	4.56	4.46	4.47	4.98	4.93	—
3	4.34	4.43	—	4.70	—	—
4	4.26	4.14	4.24	4.57	4.60	—
5	4.79	4.82	4.99	5.20	5.31	5.17
	Coliforms			<i>E. coli</i>		
	A	C	E	A	C	E
1	4.29	—	4.59	3.99	—	4.08
2	—	2.10	2.54	—	2.10	2.41
3	1.78	—	—	1.54	—	—
4	—	—	1.75	—	—	1.08
5	2.02	0	0.48	1.34	—	0

*Sample 1—before heating, 2—on reaching temperature, 3—at end of holding period, 4—from ballast tank, 5—powder.

**For treatment see Table III.

from the first run (*A*) was no doubt caused by contamination. Later tests indicated that the high pressure pump may have been the source of this contamination.

The results of the initial experiments with the flash pasteurizer are shown in Table V. By appropriately adjusting the temperature of the water jacket, the temperature of the effluent egg was varied by one-degree intervals between 55 and 62°C. The original melange contained 625,000 organisms per ml. and, with 1-ml. amounts, 10 of ten tubes were positive for coliform organisms. The initial temperature was 20° to 22°C. A 99% reduction in the viable count was accomplished with an effluent temperature of 57°C. and the reduction increased as the temperature increased. With the dilutions used, little information was obtained about the reduction in numbers of coliform

organisms; no reduction was apparent until a temperature of 62°C. was reached. However, the liquid egg leaving the pasteurizer at 61°C. was slightly thick, so 60°C. was regarded as the highest working temperature with this equipment.

TABLE V
EFFECT OF TEMPERATURE ON BACTERIAL CONTENT OF
FLASH PASTEURIZED MELANGE

Temp. egg at outlet, °C.	Viable count, no. per ml.	Coliform: no. of 10 tubes positive
55	2600	10
56	22,000	10
57	1300	10
58	590	10
59	750	10
60	150	10
61	50	9
62	25	1
Original melange	625,000	10

In a later trial the pasteurizer was set up in conjunction with the laboratory spray drier and three lots of powder dried (Table VI). Lot *K* was put through the pasteurizer, cooled immediately, and later put through the drier. Lot *L* was pasteurized and pumped directly into the drier without cooling. Lot *M*

TABLE VI
EFFECT OF FLASH PASTEURIZATION ON THE BACTERIAL CONTENT OF EGG POWDER AND ON
THE QUALITY INITIALLY AND AFTER STORAGE FOR
THREE WEEKS AT 37°C.

Lot*	Viable count, gm.	Coli, M.P.N./ gm.	Mois- ture, %	Fluorescence values		Potassium chloride value		Palat- ability
				Initial	After storage	Initial	After storage	Initial
<i>K</i>	10,000	1000	2.63	21.9	50.9	65.0	40.0	7.8
<i>L</i>	8000	0	2.25	21.5	52.0	71.0	43.0	8.8
<i>M</i>	34,000	5000	3.98	22.0	60.6	64.6	38.0	8.3

*See text for treatments.

(control) was put through the cold pasteurizer. In lots *K* and *L* the water jacket was maintained at a temperature of 62.5°C. and the egg liquid at the outlet ranged from 59.5° to 60°C.

The original melange contained 1,700,000 organisms per ml. The melange of lot *K* had a viable count of 140,000 per ml. after pasteurization (92% reduction). The data on the powders (Table VI) indicated that pasteurization

followed immediately by drying (lot L) is more effective than cooling the pasteurized liquid and drying later. However, the results are not conclusive and further work is necessary with more efficient heat exchangers.

Effect of Pasteurization on Powder Quality

The early experiments indicated that heating liquid egg for periods up to four hours did not increase the development of fluorescence during storage of the resulting powder (Table VII); the indications were that the longer heating periods retarded the development of fluorescence.

TABLE VII
EFFECT OF STORAGE FOR 21 DAYS AT 37°C. ON FLUORESCENCE VALUES
OF POWDER PREPARED FROM UNHEATED AND HEATED
LIQUID

Holding time, hr.	Fluorescence values	
	Initial	After storage
0	12.5*	29.8*
0.5	11.9	28.8
1	11.9	29.2
2	12.1	28.7
3	12.1	26.6
4	11.8	25.0
Necessary difference	1.1	2.7

*Means of three trials at 55°, 57.5°, and 60°C. The difference between them was not significant. Moisture content of all powders, $2.58 \pm 0.10\%$.

In the first commercial trial no significant differences between the powder prepared from treated and untreated melange could be detected initially by any of the measures of quality used (Table II). After storage at 36.7°C. for periods up to three weeks the fluorescence values of the pasteurized material were slightly less than those of the controls (Table II). This was attributed in part to the higher moisture content of the latter (13). As in the early experiment, there was an indication that the longer the liquid was kept hot the slower the development of fluorescence. This possibly indicates a destruction of enzymes.

The lower moisture content of the powder prepared from the heated melange is interesting. As may be seen from Fig. 1 the air outlet temperature was very high at the beginning owing to inexperience in drying hot liquid. It was necessary to reduce the heat input into the incoming air by one-half to bring the outlet temperature to the usual 135°F. As the same nozzle and the same pump pressure (2800 lb. per sq. in.) were used, no difference was observed in the amount of powder dried.

The moisture contents of the powder prepared in the second commercial trial were more comparable (Table III). The changes in fluorescence and potassium chloride values during storage at 32.2°, 26.7°, 21.1°, and 15.0°C.

of powders prepared from pasteurized melange and the control powder are shown in Fig. 2. Fluorescence measurements showed no difference between the treated and untreated material. Although average values only are shown in the figures, the potassium chloride values of the powders from unheated egg and that heated to 57°C. were slightly higher than those from egg heated to 60°C. This difference was apparent during most of the storage period but was not always noticeable at the end.

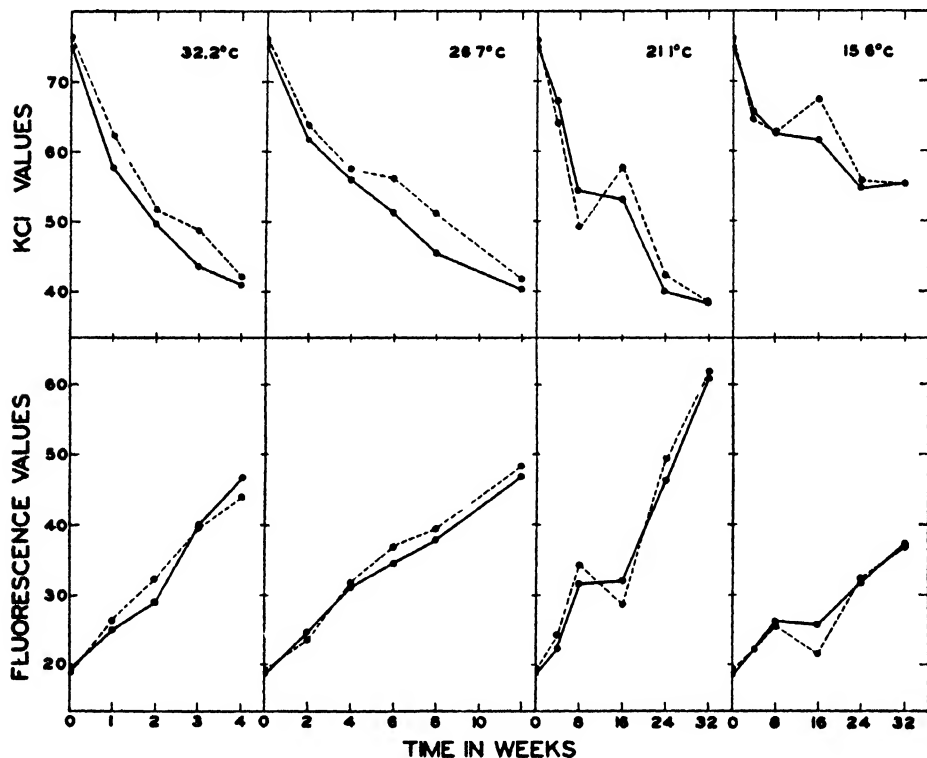


FIG. 2. Changes in fluorescence and potassium chloride values of pasteurized and unpasteurized egg powders on storage. Treatment of liquid egg as in Table III. Solid line—unheated liquid. Broken line—mean value for five lots of powder from pasteurized liquid.

The powder shipped overseas was tested there by a number of other tests (Haenni solubility, foam volume, creaming, flavour, and pH), with insignificant differences between the pasteurized powders and the control (6). It was remarked that the pasteurized powder was caked to some degree and resembled material that had been lightly compressed and then crumbled. This had been noted when packing the material but the lumps were easily broken up.

Samples of lots A and F were stored overseas at 37°C. for 10 weeks with no difference between the two in flavour, foam volume, creaming, and Haenni solubility.

With the exception therefore of a tendency to pack and a slightly lower solubility there was little or no difference in the original and keeping properties of egg powders prepared from untreated and vat pasteurized melange.

Panels of six persons passed judgment on scrambles prepared from all of the powders of the second commercial experiment before and after storage. Considering the variation between persons, there was little or no difference in the ratings given the pasteurized powders as compared with the controls. Treated and untreated powders deteriorated at practically the same rate.

The results of the initial quality tests on the powders prepared with the flash pasteurizer are shown in Table VI. There was little difference between the control (lot *M*) and the treated material. The deterioration during storage at 37°C. for three weeks was practically the same for all three powders, considering the higher moisture content of the control.

Commercial apparatus for flash pasteurization was not available for further work. However, such apparatus operating with lower temperature differentials and more turbulent flow might allow higher temperatures to be used without coagulation. Regulating the flow to drier capacity would eliminate the accumulation and holding of hot liquid, and conserve the heat introduced into the liquid.

Acknowledgments

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OIL CONCENTRATION BY THE FROTH FLOTATION OF PEBBLE-MILLED SEED¹

BY N. H. GRACE² AND J. B. PALMER³

Abstract

Froth flotation of water-cooked pebble-milled milkweed seed or mustard screenings yielded 90% of the total oil in the concentrate, which was enriched to twice the oil content of the original seed. Protein and inorganic matter also tended to concentrate in the froth fraction. Optimum conditions for separation required pH values substantially below 7 and pulp densities between 2 and 5%. Iodine numbers of oil and ease of protein peptization differed for concentrate and tailing fractions, showing some fractionation as well as concentration.

Introduction

The recent application of froth flotation to the separation of resin-rubber from milkweed leaves (5, 8, 9) suggested the further use of this method of separation or concentration of other materials occurring in plants. Seed of relatively low oil content has been selected as a plant material possibly amenable to processing with the pebble mill and flotation cell. The industrial utilization of oil from large tonnages of material such as grain screenings might be furthered by the application of comparatively low-cost methods of grinding and separating oil. This communication describes the results of preliminary experiments on the application of froth flotation to pebble-milled aqueous seed pulps. Study of the distribution of oil effected by flotation was the main object of the investigation, but some observations were also made on the distribution of protein and inorganic matter.

Materials and Methods

Seeds used were those of the common milkweed, *Asclepias syriaca* L., and screenings, largely of seeds of mustard species, family Cruciferae. Pulp was prepared for flotation by grinding one part by weight of air-dry seed (moisture content 7 to 8%) with approximately 15 parts by weight of tap water in a porcelain-lined pebble mill charged with flint pebbles. Most of the work involved cooking the seed in about 10 volumes of boiling water for a period of one hour, draining off the aqueous liquor, rinsing the drained seed with water, and then pebble-milling. A satisfactory milling period was 15 hr. with one-gallon mills, charged with 100 gm. of seed, while seven hours in a mill of 14 gal. capacity and proportionately charged, yielded pulp with approximately equal frothing characteristics. The pebble mills were drained and rinsed to remove all solid matter, and the pulp was stirred while samples

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were removed for flotation. Pulp was subjected to flotation immediately at the close of the milling period, except in one series of experiments with milkweed in which the milled pulp was held overnight.

All flotations were made in a Fagergren laboratory sub-aeration unit using a charge of 2250 ml. of pulp. Flotation at various pulp densities involved dilution of the suspensions with tap water; a range of pH values was attained by addition of sodium hydroxide or sulphuric acid. The suspension was agitated for a period of two minutes at room temperature (about 20° C.) prior to aeration. Termination of the flotation could be judged by the increase in bubble size, the usual period being from three to four minutes. No flotation reagents were added, since the pulp contains its own frothing and collecting reagents. The concentrates (fraction carried over by froth) and tailings (residual material) were rinsed into bottles with a minimum amount of water. Samples of the feed (pulp fed to float cell) also were preserved for analysis.

The bottled suspensions of feeds, concentrates, and tailings were then centrifuged in 250-ml. wide-mouth tubes at approximately 2000 r.p.m. The liquors were usually clear, though recentrifuging was required at some of the higher pH levels. The cakes were dried *in vacuo* at a temperature no higher than 40° C., and ground in a Wylie mill. Total dry weights were recorded and samples of about 5 gm. subjected to extraction in a Soxhlet apparatus with petroleum ether (b.p. 30 to 60° C.) for a period of 24 hr. Determination of the weights of oil from cake or ground seeds permitted calculation of oil contents.

All analytical data are presented on a moisture-free basis. Protein (% Kjeldahl nitrogen $\times 6.25$) and ash percentages are based on the weight of oil-free cake. Pulp densities are referred to dry weight of water-extracted seed. The pH values are given as recorded by a Coleman glass electrode. Iodine numbers (Wijs) were determined for a few of the mustard oil samples. The nitrogenous constituents of a few samples of solvent-extracted mustard meal were peptized by a recently described procedure (4).

Data are given in graphical form for the per cent of oil, protein, or inorganic matter in both concentrates and tailings. Similar data are given for distribution to the concentrates, expressed as a percentage of the total amount of material, e.g., oil, obtained from both fractions.

Analyses of Seed and Pulps

The oil contents of these milkweed seeds and mustard screenings were, respectively, 22.3 and 21.8%. Water digestion prior to pebble-milling removed approximately 15% of water-soluble materials. The oil contents of this water-extracted seed for both milkweed and mustard were of the order of 25 to 26%.

Attainment of analytical balance of total solids and oil was an obvious prerequisite of any study of flotation. While the pebble-milling operation was effective in grinding, it tended to emulsify a part of the finely ground material. It was found that solid matter from centrifuged feed samples had

oil contents closely approximating those of the water-extracted seed before pebble-milling. However, if the prepared pulp was held for several hours between the end of the grinding operation and the beginning of flotation, the oil content of feed samples tended to be a little greater, owing, apparently, to the gradual solution of part of the material. Centrifuging of feed samples, over a range of solid contents of from 1 to 5%, recovered from 90 to 98% of both total solids and oil. Recovery approximated 100% at low pH values but fell to about 90% at pH values substantially above 7.

The violent agitation of pulps in the flotation cell might be expected to effect further emulsification and render subsequent centrifugal separation of solids and oil difficult. Consequently, the effects of pulp density and pH on recovery after flotation were investigated. The amounts of solid and oil from concentrates and tailings were obtained separately, but the combined values were expressed as percentages of the amounts recovered from feed samples.

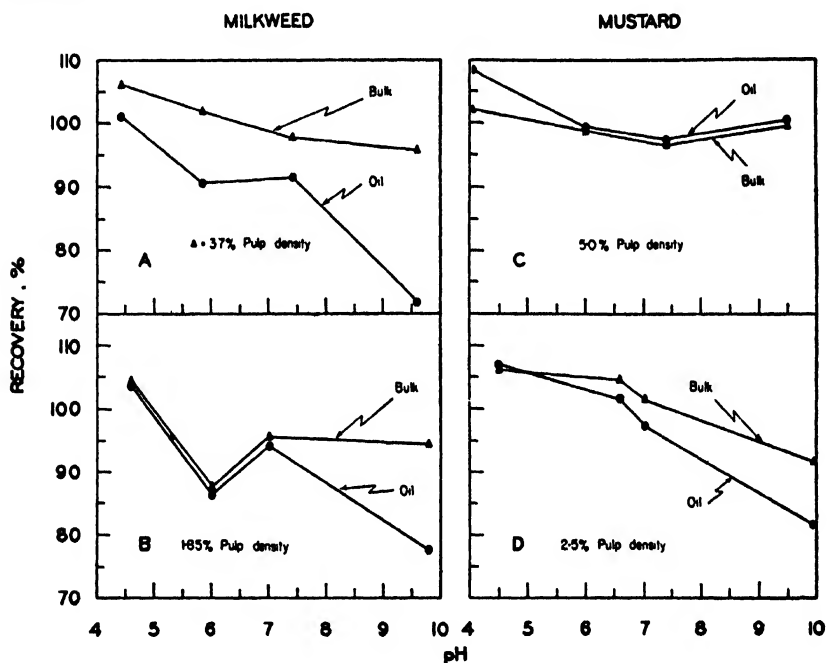


FIG. 1. Recovery of bulk and oil after flotation at different pH levels as a percentage of recovery from feed. (Milkweed and mustard feeds, respectively, at pH levels of 5.8 and 6.0.)

The graphs in Fig. 1 show the effects of pH on recovery of total solids and oil from milkweed and mustard seed, each at two pulp densities. Since milkweed and mustard seed when pebble-milled in tap water yield pulps with pH values of 5.8 and 6.0, respectively, these were the pulps chosen as reference feed samples. Recoveries of above 100% are accounted for by the greater efficiency of centrifugal separation in the pH range around 4 as compared

with recoveries from feed samples at pH 5.8 to 6.0. It is apparent that pH has a pronounced effect on recovery. The disadvantageous effects of high pH are more marked at lower pulp densities and on the oil fraction. Oil recovery does not fall to 90% over the entire pH range for mustard with pulp density of 5% (Fig. 1,C). Reduced recovery is indicated in the isoelectric region for milkweed (Fig. 1,B). These results show that oil recovery of about 100% is attained for pulps floated at pH values of 5 or less.

The effects of milkweed seed pulp density at pH about 5.8 on recovery from concentrates and tailings as a per cent of feed at the same pH and density are described in Fig. 2. The violent agitation of flotation of pulp with density of 6% is followed by better recovery than for the feed. Recovery increased approximately linearly with increasing pulp density; oil recovery showed more sensitive response than bulk throughout.

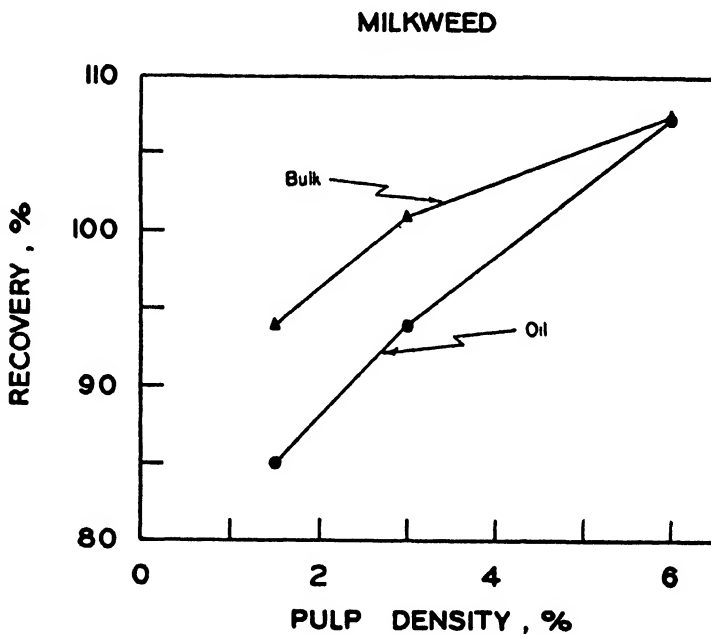


FIG. 2. Recovery of bulk and oil after flotation at different pulp densities as a percentage of feed under corresponding conditions (pH approximately 5.8).

It is apparent from these results that the analytical procedure yields recoveries that closely approach 100% either in the pH range below 5 or at pulp densities above 5%. In view of these findings, further refinement of analytical methods for this preliminary investigation was not deemed warranted. However, it must be recognized that the following results on flotation at either high pH values or low pulp densities are based on recovery of somewhat less than 100% of the material initially present.

Results

Frothing Characteristics

Pulps prepared from raw milkweed and mustard seed frothed voluminously, with resulting poor separation; pulps from seed previously cooked in water frothed less and showed appreciable concentration of oil in the froth fraction. Consequently, only a few preliminary observations were made on raw seed and the main investigation was confined to seed that had been cooked in water. Both pulp density and pH affected the frothing characteristics. Under favourable conditions numerous small, heavily laden bubbles were formed and the froth was wet. Froth became excessively watery under alkaline conditions; appreciably better frothing was noted with acid pulps. The frothing behaviour was quite similar to that of milkweed and other leaf tissue (9).

Effects of Pulp Density

The effects of froth flotation on oil content and distribution of oil for milkweed and mustard seed pulps with pH values of 5.4 to 5.8 but over a range of pulp densities are shown in Fig. 3. The oil content of the concentrate varied little over the range of pulp density. However, the oil contents of milkweed tailings tended to rise with increasing pulp density, while those of mustard

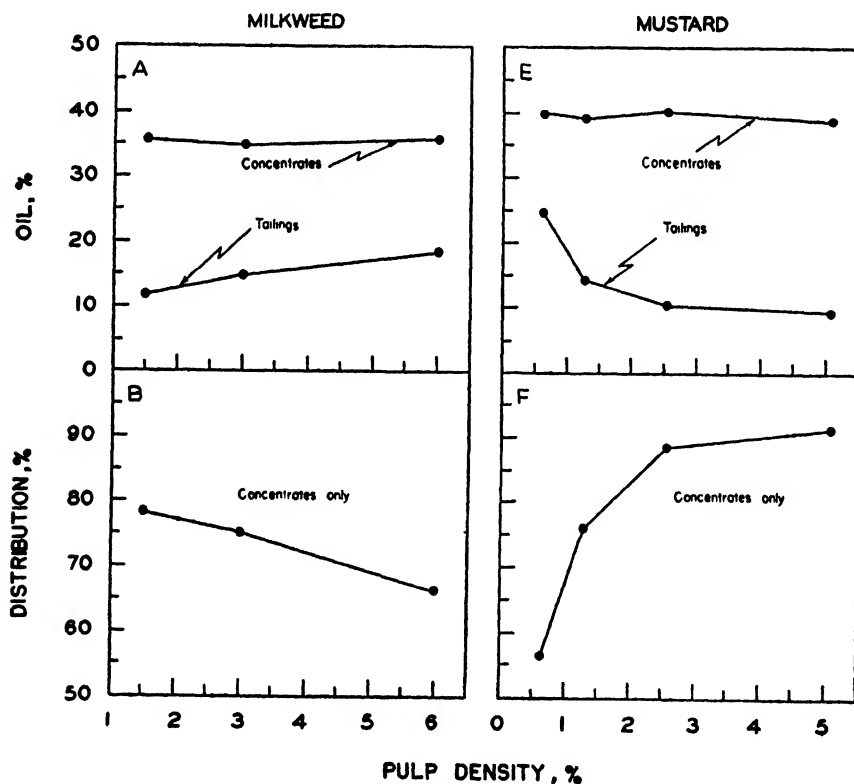


FIG. 3. Effects of pulp density at pH approximately 5.6 on content and distribution of oil following froth flotation.

fell rapidly and became constant. A similar tendency was noted for oil distribution in the concentrates, milkweed showing a greater percentage at low pulp densities and mustard at high. It is apparent that substantial concentration of oil occurred in the fraction carried over by the froth.

Effects of pH

In Fig. 4, *C, D*, are described the effects of pH on oil content and distribution of oil for milkweed. There was substantial oil concentration over the whole

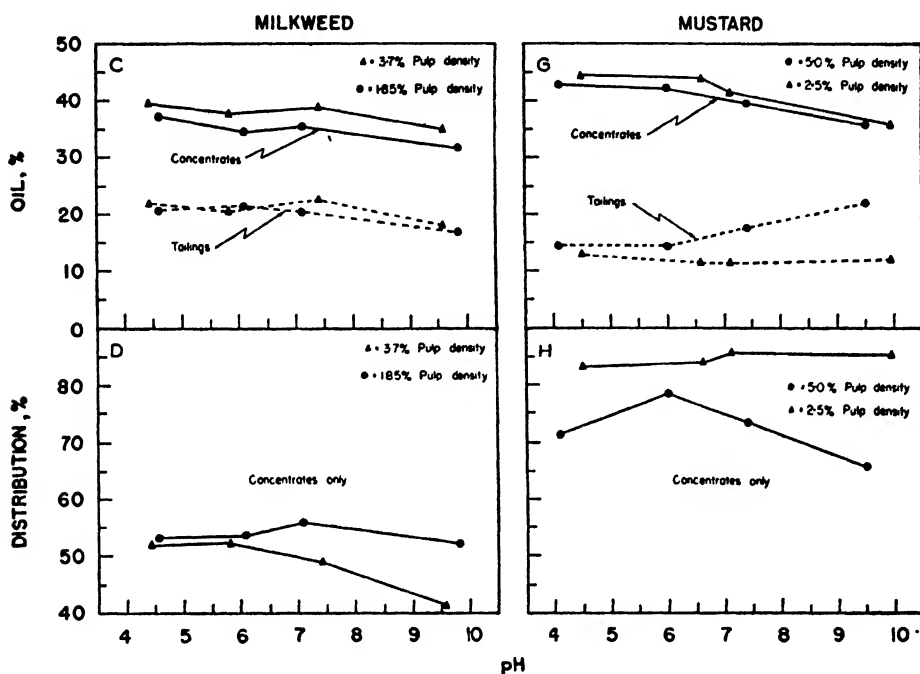


FIG. 4. Effects of pH levels on content and distribution of oil following froth flotation.

pH range. The oil content at 3.7% pulp density was fairly uniform but slightly greater than for pulps of one-half this density. The oil content of the tailings was approximately equal throughout for both densities. The relatively high oil contents of these tailings (compare with Fig. 3, *A*) may be attributed to the fact that the pebble-milled pulp stood overnight between milling and flotation, after which the oil contents of feed samples approximated 30% instead of the usual value of 26%. It may be seen from Fig. 4, *D* that the concentrates contained from 50 to 60% of the total oil for pulps of 1.85% solids, with a decline indicated at high pH values. This decline was accentuated with increasing pH for the pulp of greater density.

The oil content and distribution for mustard seed pulps are shown in Fig. 4, *G, H*. It is apparent that the results are similar to those described for milkweed though a much higher percentage of the total oil is found in the concentrates. It is, however, of interest to note the approximate linearity

of the curve for distribution of oil in the concentrates at 2.5% solids, also the tendency towards convergence at high pH values for the curves describing oil content of concentrates and tailings.

Distribution of Inorganic Matter

The ash from water-extracted mustard screenings amounted to 7.04%; that recovered from the pebble-milled pulp was 16.5%. This substantial increase in ash content resulted from attrition of pebbles and mill-linings. The effects of flotation on ash content and distribution over a range of pH values are described by the graphs of Fig. 5, A, B. It is apparent that the froth

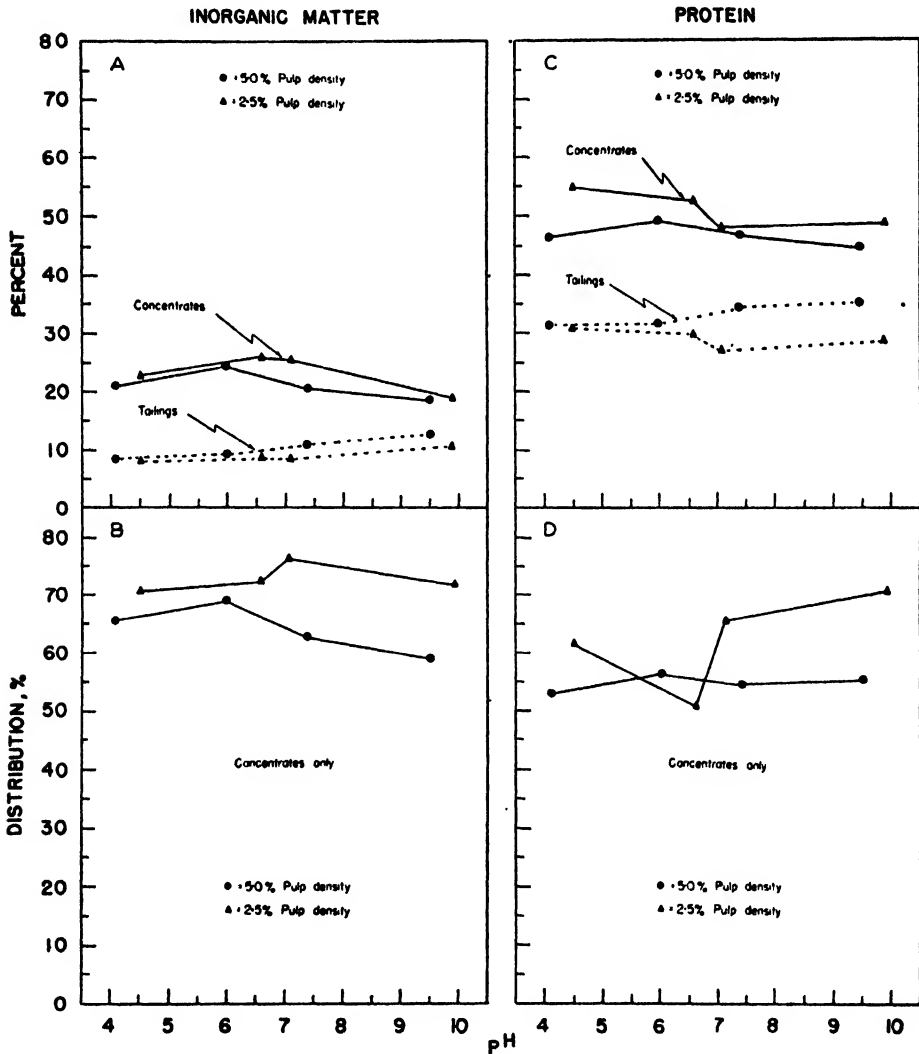


FIG. 5. Effects of froth flotation at different pH levels on content and distribution of inorganic matter and protein of mustard seed.

has been substantially enriched in mineral matter, a maximum occurring in the pH range from 6 to 7. The ash content of concentrates and tailings tended to converge at pH values of 9 to 10, this effect being more pronounced at the higher pulp density. From 70 to 75% of the total inorganic matter of the more dilute pulp was distributed to the concentrates over the pH range. Appreciably less inorganic matter was found in the concentrates at the higher pulp density, and the reduction effected by high pH values was accentuated.

Protein Distribution

The protein content of water-extracted mustard screenings was approximately 43%, while feed samples showed values between 40 and 41%. The graphs in Fig. 5, C, D, describe the per cent of protein and its distribution in the concentrates over a range of pH values. There was substantial concentration in the froth, which was more pronounced at the lower pulp density. While high pH values tended to reduce protein content of the froth the actual amount distributed thereto was not reduced. Minima in the graphs for distribution of protein to the froth fraction are indicated, the lower pulp density being more affected in this respect.

Calculation of protein content on an ash-free basis results in the graphs in Fig. 6. The comparable protein contents of feed at pH 6 were 49 to 50%. The inflections in the graphs indicated for distribution and protein content at the lower pulp density, Fig. 5, C, D, are greatly accentuated when data

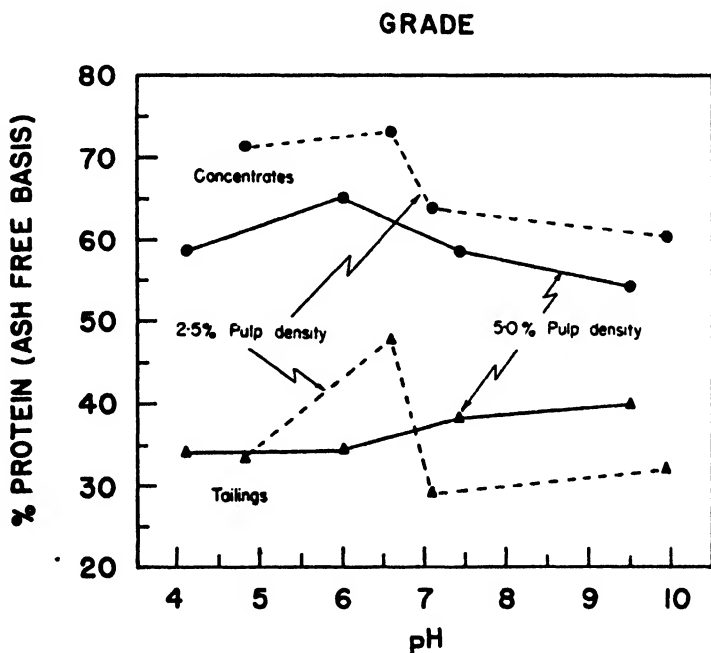


FIG. 6. Effects of froth flotation at different pH levels on protein content (ash-free basis) of mustard seed.

are expressed on an ash-free basis. It is apparent that the protein contents of concentrates and tailings were greatly affected by both pH and pulp density.

Effects of Flotation on Quality of Oil and Protein

Oils from concentrates tended to be darker and less viscous than oils from tailings. However, the pH at which flotation occurred influenced the extent of these differences and the iodine number of the oil. Oil from mustard pulp floated at pH 6 gave iodine numbers of 125 and 88 for concentrates and tailings, while corresponding values for flotation at pH 7.4 were 115 and 123. The iodine number of oil from feed was 119, a value approximately equal to that from the weighted averages of oils obtained by flotation at the specified pH levels.

Somewhat similar effects were noted for quality of mustard protein from concentrate and tailing fractions as judged by the degree of dispersion. Concentrates obtained in flotation under acid conditions yielded protein that was more readily dispersed than that of the corresponding tailings. However, flotation at pH 7.4 showed a tendency for more of the readily peptized material to occur in the tailings. The method of dispersion (4) specified a peptization period of three hours, which was found inadequate for mustard. Consequently, these results may be considered indicative but not conclusive. They do, however, suggest that some protein fractionation, as well as concentration, occurred concomitantly with oil concentration.

Discussion

These preliminary results show that oil-enriched concentrates result from the application of froth flotation to slurries from pebble-milled seeds. The judicious selection of pulp density and pH of flotation results in up to 90% of the oil occurring in the concentrates, which have an oil percentage approximately double that of the original seed. The distribution of inorganic and nitrogenous materials is also affected by flotation. The differences noted in iodine number of the oil and peptization tendency of the protein also call for comment. These results suggest that flotation, in addition to its use for effecting concentration, may lead to a measure of controlled fractionation. Study of gases other than air would be interesting in this connection. Earlier workers have effected some fractionation of the sodium salts of a mixture of fatty acids, using streams of carbon dioxide or nitrogen (3).

While froth flotation apparently has not been applied previously to the processing of oil from seeds, it has been used in the separation of wool-fat from wool washing effluent (1, 2). It is of interest to note that seed slurries contain their own frothing and collecting reagents, being similar in this respect to other preparations from plant materials (9). The marked effects of protein and inorganic matter on flotation suggest that frothing may relate to the combined effects of oil, inorganic matter, and protein.

This investigation has been limited to a study of the applicability of froth flotation to the production of fractions enriched in oil. Development of methods for the extraction of oil from concentrates has been beyond the scope of the investigation. However, successful integration of pebble-milling and flotation of seed pulps into a feasible oil extraction process depends on removal of oil from the concentrate. Recently described methods for oil extraction (6, 7) involving pebble-milling and centrifuging could, it is believed, be simplified and improved by the inclusion of froth flotation.

Acknowledgments

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THE APPLICATION OF DUST-LAYING OIL TO COTTON¹

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Abstract

Details of a process for the application of dust-laying oil to cotton fabric are given. The process consists essentially of the mutual coagulation, in the presence of the cotton, of two oppositely charged oil-in-water emulsions. The negative and positive emulsions are stabilized with commercial preparations of sodium ethyl sulphonate methyl-oleamide and cetyldimethylbenzylammonium chloride respectively. The ratio of the two emulsifying agents must be controlled accurately in order to ensure complete coagulation of the oil.

The uniformity of oil distribution on the fabric is satisfactory, and there is little or no tendency for fatty acid or oil to build up on repeated laundering and oiling when carried out in a commercial laundry wash wheel. There is some loss of oil owing to deposition on the wheel, so that it is necessary to allow for 7% oiling in order to obtain 4 to 6% on the fabric.

Introduction

The application of dust-laying oil to hospital bed-clothes for the purpose of reducing dust-borne infection has been the subject of considerable study during the past few years (6, 9, 10, 11). The theoretical and practical aspects of the application of oil to woollen fabrics have already been discussed by Bayley and Weatherburn (3) and by Bayley, Rose, and Weatherburn (2). It is the purpose of the present paper to deal in a similar way with the application of oil to cotton fabrics.

The work is based on the method of Harwood, Powney, and Edwards (6), in which the oiling of cotton fabrics is accomplished by the mutual coagulation, in the presence of the cotton, of two oppositely charged oil-in-water emulsions.

Experimental

Materials Used

A technical white oil, Marcol HX, supplied by Imperial Oil Company, was used in the preparation of the emulsions.

Some preliminary work was carried out using negative emulsions (i.e., emulsions in which the oil drops bear negative charges) stabilized with sodium lauryl sulphate (Duponol WA). It was found however that these emulsions creamed very rapidly and made accurate measurement difficult. Consequently a survey was made of a number of commercially available anionic surface active agents. The stability of emulsions formed with these agents (Table I) was determined in the following manner. The weight of compound indicated in the table was dissolved in 50 ml. of hot, distilled water, 20 gm. oil was stirred in, the volume was made up to 100 ml. with distilled water, and the mixture was passed through a colloid mill. The emulsions were stored in stoppered

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TABLE I
ANIONIC SURFACE ACTIVE AGENTS INVESTIGATED

Reference No.	Name	Type	Manufacturer	Weight used in stability test, gm.
1	Igepon T	$C_{17}H_{33}CONCH_2C_2H_4SO_3Na$	General Dyestuff Corp.	1
2	Igepon AP Extra	Sodium sulphonate of oleic acid ester of aliphatic alcohol	General Dyestuff Corp.	1
3	Igepal C	Polymerized ethylene oxide condensate	General Dyestuff Corp.	1
4	Naccolene F	Modified alkyl aryl sulphonate	National Aniline Division of Allied Chemical and Dye Corporation	1
5	Calsolene Oil HS	Highly sulphonated oil	Imperial Chemical Industries	1
6	Teepol	Sodium secondary alcohol sulphate	Shell Oil Co.	3
7	Aerosol OS	Isopropyl naphthalene sodium sulphonate	American Cyanamid and Chemical Corporation	1
8	Duponol WA	Sodium lauryl sulphate	E. I. du Pont de Nemours Co.	1.5
9	Duponol WA	Sodium lauryl sulphate	E. I. du Pont de Nemours Co.	0.75
10	Nacconol NRSF	Sodium alkyl aryl sulphonate	National Aniline Division of Allied Chemical and Dye Corporation	1
11	Santomeræ No. 3	Alkylated aryl sulphonate	Monsanto Chemical Co.	1
12	Aerosol OT	Diocetyl ester of sodium sulphosuccinic acid	American Cyanamid and Chemical Corporation	1

bottles at room temperature for a period of two months. At the end of this time the bottles were shaken thoroughly and allowed to stand undisturbed overnight. The appearance of the emulsions on the following morning is shown in Fig. 1.

Breakdown of the emulsion with the separation of clear oil is apparent in those emulsions stabilized with Igepon AP Extra (No. 2) and with Naccolene F. (No. 4). With these two exceptions the emulsions have been arranged in order of their tendency to cream, i.e., to separate into two layers, the upper one of which contains a high proportion of oil, while the lower one is largely water. This property does not prohibit the use of these emulsions in the oiling process, since the oil is readily redispersed by gentle mixing, but it does tend to make accurate measurement more difficult, and, as will be shown later, accuracy of measurement is of considerable importance in the oiling of cotton by the proposed method.

The majority of the emulsions foamed excessively on milling. This property, while not objectionable in so far as the oiling process is concerned, might present some difficulty in the preparation of emulsions on a large scale. The compounds are divided into three groups according to their tendency to foam on violent agitation.

Group A (Very little foaming)

Igepon T
Igepon AP Extra
Igepal C

Group B (Moderate foaming)

Naccolene F
Calsolene Oil HS

Group C (Excessive foaming)

Teepol
Aerosol OS
Aerosol OT
Duponol WA
Nacconol NRSF
Santomerse No. 3

It is apparent that Igepon T shows the greatest promise from the standpoint of emulsion stability and foaming properties. This compound was therefore selected for further study.

Triton K-60 (cetyldimethylbenzylammonium chloride) was used in the preparation of the positive emulsion.

The cotton sheeting used had a thread count of 76 by 67 threads per inch and a weight of 4.6 oz. per sq. yd. For the laboratory experiments the cotton was freed from water-soluble sizing materials by immersion for one hour in boiling distilled water, followed by a second one hour immersion in boiling water and three rinses in cold water. This desizing treatment was omitted on the cotton used for the pilot plant trial.

Distilled water was used for all of the laboratory work, while for the pilot plant trial Ottawa city water, having a hardness of 4.5 to 5.0 grains calcium carbonate per Imperial gallon, was used.

Preparation of Type A Emulsions

Two stock emulsions, designated "Type A," were prepared as follows.

Material	Negative emulsion	Positive emulsion
Oil, gm.	200	200
Igepon T, gm.	20	—
Triton K-60, gm.	—	20
Water (65° C.), ml.	742	742

PLATE I

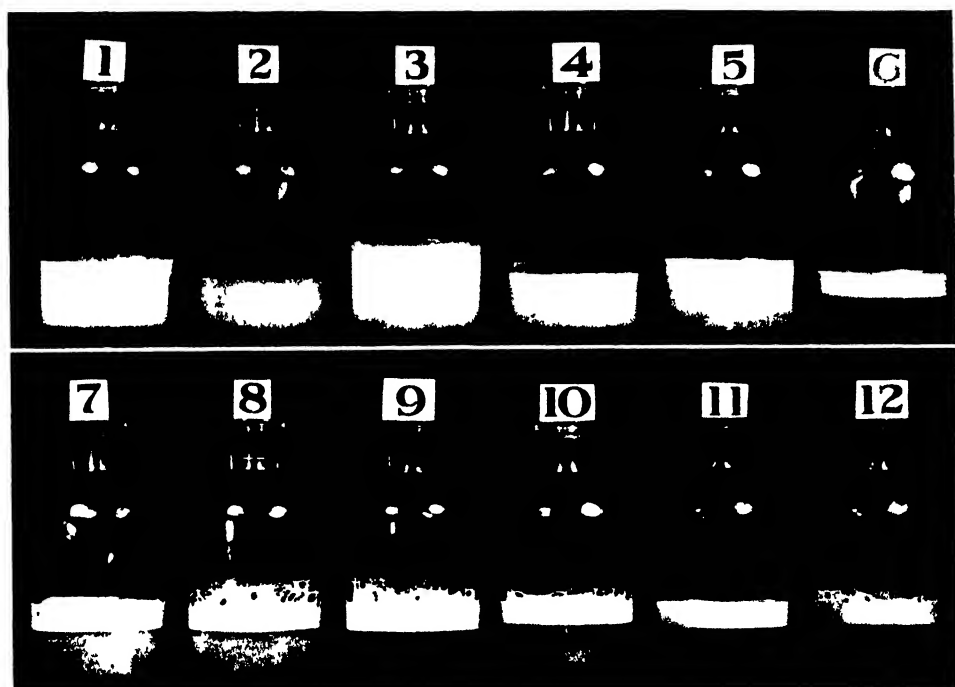


FIG. 1 *Emulsion stability test*

The emulsifying agent was dissolved in the hot water, the oil was stirred in, and the mixture was passed through a colloid mill. Better emulsions were obtained with Igepon T when the oil was heated to 65° C. before mixing. This was not necessary in the case of the Triton K-60 emulsion. The emulsions were found by analysis to contain 20.0 ± 0.2 gm. oil per 100 ml.

Adsorption of Oil by Cotton

It is well known that cotton, when immersed in water, acquires a negative charge (4, 5, 8). It is to be expected therefore that the adsorption of oil by cotton will be greater from a positively charged emulsion than from one that is negatively charged. This fact was confirmed by carrying out experiments similar to those previously described for the measurement of the adsorption of oil by wool (3). A 10 gm. sample of cotton, cut into small pieces, was agitated in a mixture of 200 ml. of water and 10 ml. of Type A negative emulsion at 40° C. Samples of the emulsion were withdrawn for analysis at intervals of 5, 10, 20, and 40 min. The experiment was repeated using 10 ml. of Type A positive emulsion. The results are given in Fig. 2.

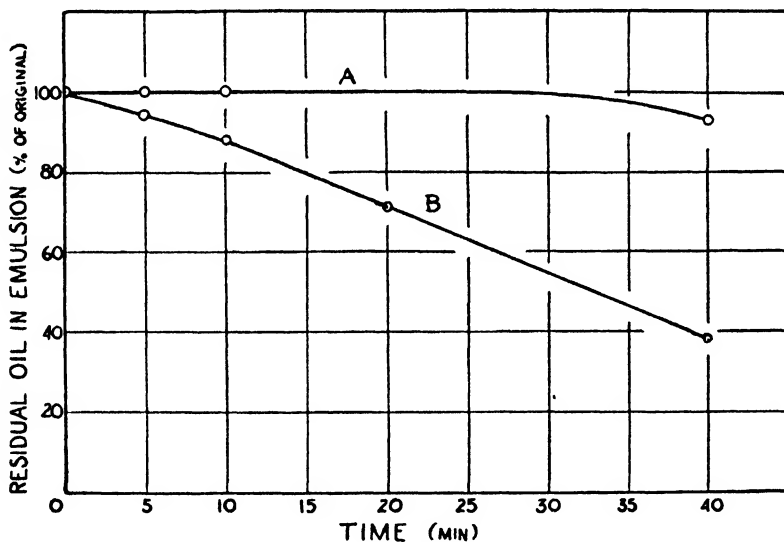


FIG. 2. Adsorption of oil by cotton. A—Negative emulsion; B—positive emulsion.

Balance Test

Since the electrical attraction of the cotton for either one of the emulsifying agents is not sufficient to cause complete exhaustion of the treating bath, the deposition of oil on the fabric must be accomplished by the mutual coagulation of two oppositely charged emulsions. It follows that the two emulsifying agents must be present in the proper proportions to bring about complete coagulation of the oil. If either is present in excess, some oil will remain dispersed, and complete exhaustion of the bath is impossible. In order to

determine the optimum ratio of the two emulsifying agents the following "balance" test was devised.

To each of seven test tubes containing 5 ml. of Type A positive emulsion was added an amount of negative emulsion varying from 2 to 5 ml. in increments of 0.5 ml. The tubes were stoppered, shaken, and allowed to stand. A marked coagulation was evident in some of the tubes within a few minutes, and after one hour the contents of these tubes had separated into two distinct layers, the lower of which was practically clear water. The contents of the remaining tubes remained milky in appearance. The tubes that showed rapid coagulation were those containing from 3.0 to 4.5 ml. of the negative emulsion, i.e., the optimum ratio of negative to positive emulsifying agent is approximately $\frac{3.75}{5} = 0.75$, and the "usable range" is from about $\frac{3}{5} = 0.6$ to $\frac{4.5}{5} = 0.9$.

In order to check the validity of these conclusions a series of oiling experiments was carried out as described in the preceding section. The 10 gm. sample of cotton was agitated for two minutes in a mixture of 200 ml. of water and 3.5 ml. of Type A negative emulsion at 40° C. Then 7 ml. of positive emulsion was added (ratio 0.5) and the stirring continued. Samples of the

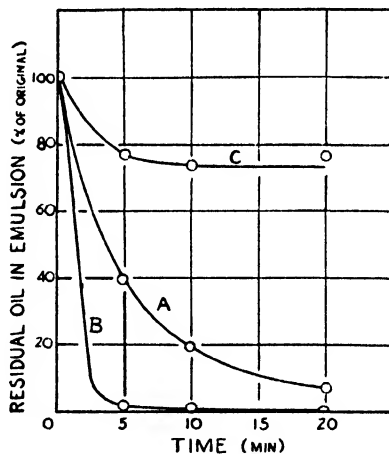


FIG. 3. Effect of emulsifying agent ratio on oil adsorption by cotton. A—Ratio 0.5 (excess positive); B—ratio 0.75 (optimum); C—ratio 1.0 (excess negative).

bath were withdrawn for analysis at 5, 10, and 20 min. intervals, the time being measured from the addition of the positive emulsion. The experiment was repeated with negative : positive ratios of 0.75 and 1.0, keeping the total volume of emulsions used constant. The results, calculated as percentages of the total oil used, are given in Fig. 3.

It is apparent that the balance test gives a satisfactory indication of the performance to be expected in the actual oiling operation. The importance

of selecting the correct ratio between the two emulsifying agents is also emphasized.

Order of Addition of Emulsions

Harwood, Powney, and Edwards (6) recommend that the positive emulsion be added first, followed by the negative. In the preliminary work with Duponol WA there was some indication that more rapid exhaustion of the bath is obtained if this order is reversed. This has been confirmed with Type A emulsions. The oiling procedure described in the preceding section was repeated using a ratio of 0.75 but reversing the order of addition of the emulsions. The results are given in Fig. 4. In plotting these figures the

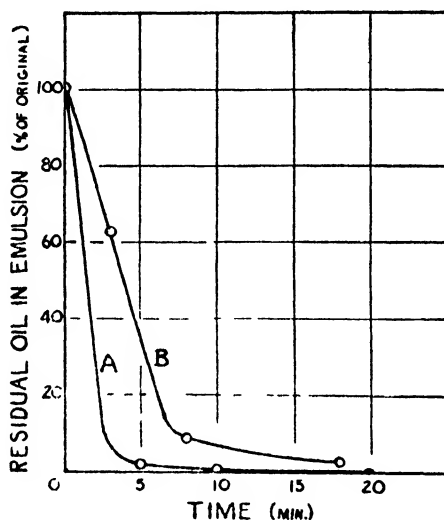


FIG. 4. Effect of order of addition of emulsions on oil adsorption by cotton. A—Negative emulsion added first; B—positive emulsion added first.

small amount of oil adsorbed by the cotton from the positive emulsion, prior to the addition of the negative emulsion, has been neglected. The initial point on this curve should obviously be slightly less than 100%.

It is apparent that the deposition of oil is more rapid when the negative emulsion is added first, followed by the positive. This is probably explained by the fact that cotton shows a much stronger selective adsorption for the positive emulsifying agent than for the negative (see Fig. 2). When the positive emulsion is added first, the cationic agent is partially adsorbed by the cotton, with the result that the ratio between the two emulsifying agents is upset, and there is, in effect, an excess of negative emulsifying agent present. When the negative emulsion is added first there is little or no selective adsorption by the cotton, and consequently the ratio between the two emulsifying agents is unchanged.

Influence of pH of the Emulsions

The influence of the pH of the emulsion on the rate of adsorption of oil by cotton was investigated for both negative* and positive emulsions. Within the range studied (pH 3.1 to pH 8.3) it was found that the rate of exhaustion of the oil-bath decreased with increasing pH of the negative emulsion, and increased with increasing pH of the positive emulsion. It has been shown (7) that the magnitude of the negative charge on cellulose increases in alkaline solution and decreases in acid solution. This variation in the magnitude of the charge on the cotton results in varying degrees of attraction for the positive emulsion and of repulsion for the negative emulsion, and undoubtedly accounts for the pH effects noted above.

The pH values of the two emulsions were: negative—6.8; positive—4.0. Adjustment of the pH does not result in any advantage from the standpoint of total oil adsorbed, and hence, in subsequent work, the pH was not adjusted.

Preparation of Type B Emulsions

In the wool oiling process (2) emulsions containing 70% oil and 1.75% Triton K-60 by weight have been found satisfactory. It was found, however, that 70% oil emulsions are not readily formed with Igepon T, and therefore the oil content of the negative emulsion was reduced to 60%. The concentration of Igepon T was reduced to 1.32% so that the two emulsions would contain equivalent amounts of negative and positive emulsifying agents. $\left(\frac{1.32}{1.75} = 0.75, \text{ the optimum ratio}\right)$.

The composition of these emulsions was as follows:

Material	Negative emulsion, % by weight	Positive emulsion, % by weight
Oil	60.00	70.00
Igepon T	1.32	—
Triton K-60	—	1.75
Water	38.68	28.25
	100.00	100.00

The method of preparation was the same as that described for Type A emulsions. These emulsions were designated "Type B." They differ from Type A not only in the increased oil content but also in the ratio of emulsifying

* This work was carried out using a negative emulsion stabilized with Duponol WA, during the preliminary stages of the research. The conclusions reached should apply equally well to emulsions stabilized with Igepon T.

agent to oil, which has been reduced from 10% in the Type *A* emulsions to 2.2% in the negative, and 2.5% in the positive Type *B* emulsion.

In order to test further the stability of these emulsions, a series of balance tests was carried out on both Type *A* and Type *B* emulsions at intervals over a period of two months. The Type *B* emulsions were diluted (150 gm. made up to 500 ml. with water) in order to make the oil content approximately the same as that of the Type *A* emulsions. The essential difference between the two pairs of emulsions was therefore in the amount of emulsifying agent. The final composition of the four emulsions (in gm. per 100 ml.) was as follows:

	Type <i>A</i>		Type <i>B</i>	
	Negative	Positive	Negative	Positive
Oil	20	20	18	21
Emulsifying agent	2	2	0.40	0.53

Typical balance tests are illustrated in Figs. 5 and 6. Each tube shown in Fig. 5 contained 5 ml. of positive, and 2 to 5 ml. of negative Type *A* emulsion, while each tube shown in Fig. 6 contained 5 ml. of positive, and 3 to 6 ml. of negative Type *B* emulsion.

The optimum ratio was calculated as before from the mid-point of the coagulation range, and the "usable range" from the two extremes. There were slight variations in the test from day to day, possibly owing to difficulty in obtaining homogeneous samples, or to small inaccuracies in measurement, but there was no indication of a consistent change in the properties of the emulsions during the two month period. On averaging the results of all of these tests it was found that the optimum ratio was 0.74 for both types of emulsions, but that for the Type *A* emulsions the "usable range" was 0.6 to 0.9, whereas for Type *B* the "usable range" was only 0.72 to 0.76.

It may be concluded that when Type *B* emulsions are used, they must be balanced accurately in order to obtain complete deposition of the oil. When larger amounts of emulsifying agents are used (e.g., Type *A* emulsions) satisfactory oiling can be obtained over a much wider range of concentrations, and consequently more latitude is allowable in the preparation and measurement of the emulsions.

Laboratory Scale Trial of Process

In order to carry out the complete process on a small scale, use was made of a Launderometer (1). Samples of cotton sheeting weighing approximately 10 gm. were laundered and oiled according to the following formula.

Operation	Material	Temperature, °F.	Time, min.
1. Suds	150 ml. built soap solution	160	10
2. Suds	150 ml. built soap solution + bleach	160	10
3. Rinse	150 ml. water	160	3
4. Rinse	150 ml. water	160	3
5. Rinse	150 ml. water	160	3
6. Sour	150 ml. water + 4 ml. 0.75% Na_2SiF_6 solution	120	3
7. Oiling	(a) 150 ml. water + 5 ml. negative emulsion	120	2
	(b) 5 ml. positive emulsion	120	8

Note:—The built soap solution contained 0.3% neutral soap and 0.1% sodium metasilicate. In the bleaching operation, sodium hypochlorite was added to give a concentration of approximately 0.01% available chlorine.

The pH of the solution at the conclusion of the souring operation was 3.60 to 3.75.

Type B emulsions were diluted so that 5 ml. of negative + 5 ml. of positive contained sufficient oil to give approximately 7% oil on the cotton.

At the conclusion of the oiling operation, the samples of cotton were removed, allowed to drain into the jars, and dried in air. The oil content was determined as described previously (2). The residual bath was poured from the jars and its total oil content was determined. It was observed that some oil had deposited on the sides of the jars. Consequently these were rinsed with isopropyl alcohol, followed by petroleum ether, and the oil content of the rinsings was determined separately. The amounts of oil recovered from the fabric, from the residual bath, and from the sides of the jars are each expressed as a percentage of the total oil used. The oil not accounted for in this way is recorded in the "Loss" column.

The above tests were carried out using (a) the complete formula, (b) the complete formula with the omission of the bleach, and (c) the oiling operation only. From the results obtained (Table II) it may be observed that (a)

TABLE II
OIL RECOVERY IN LAUNDEROMETER TRIAL

Treatment	Oil recovered, % of oil used				Oil on fabric, % by weight
	On fabric	In residual bath	On launderometer jar	Loss	
Complete formula	77.3	3.2	6.3	13.2	4.9
	81.5	2.8	6.0	9.7	4.9
Complete formula omitting bleach	81.5	2.5	4.8	11.2	5.3
	84.8	3.0	6.4	5.8	5.2
	78.6	2.4	4.8	14.2	5.2
Oiling only	78.7	5.1	8.9	7.3	4.9
	77.1	2.9	5.5	14.5	4.9

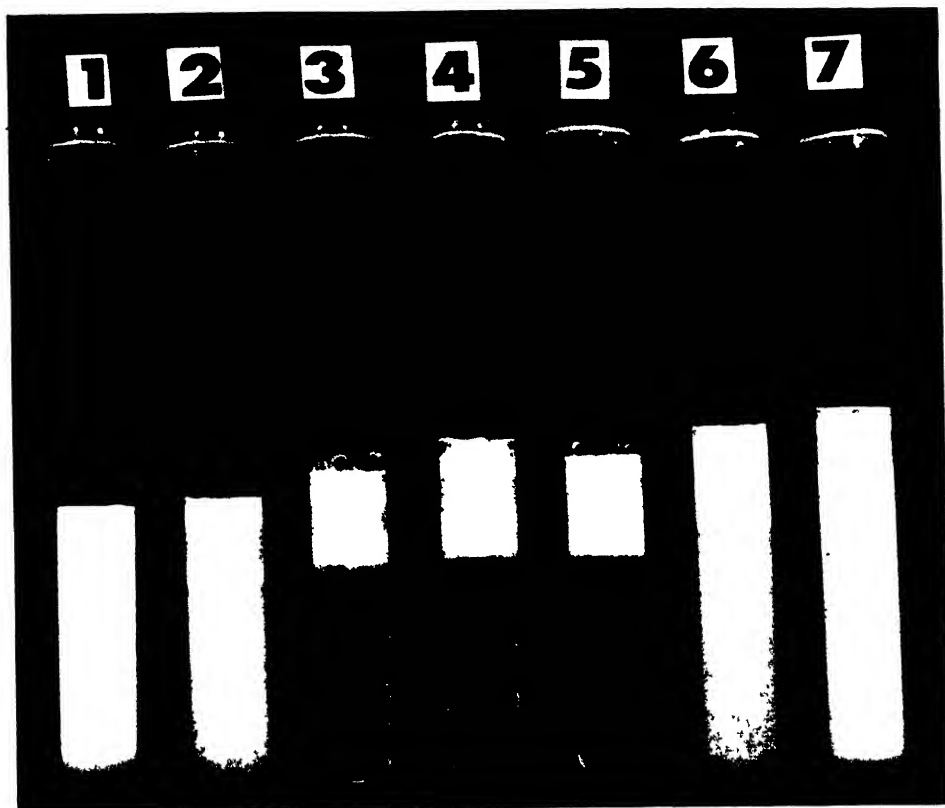


FIG. 5 Balance test. *Type A emulsions.*



FIG. 6 Balance test Type B emulsions

approximately 80% of the oil used is retained by the fabric, (b) the preliminary laundering, with or without the use of bleach, has no effect on the oiling process, and (c) from 4 to 9% of the oil used is deposited on the sides of the containing vessel.

Pilot Plant Trial of Process

For the purpose of carrying out a trial on a larger scale, a 24 by 24 in. monel metal commercial laundry wash wheel was used. The 15 lb. load of cotton sheeting was laundered and oiled according to the following formula.

Operation	Material	Water level, in.	Temperature, F.	Time, min.
1. Suds	Built soap solution	3	160	10
2. Suds	Built soap solution	3	160	10
3. Rinse	Water	7	160	3
4. Rinse	Water	7	160	3
5. Rinse	Water	7	160	3
6. Sour	Sodium silicofluoride	7	120	10
7. Oiling	(a) Negative emulsion	7	120	2
	(b) Positive emulsion	7	120	8

Note:—The built soap solution contained 10% neutral soap and 3 3% sodium metasilicate. Approximately 1.5 litres was required for each suds.

The sour was added in the dry form, approximately 50 gm. per load being required to reduce the pH to 4.5–5.0. The souring time could have been reduced to three to five minutes by adding the sour as a 1% aqueous solution.

Type B emulsions were diluted so that 1 litre of each was required to give 7% oil on the load.

After oiling, a sample of the residual bath was removed for analysis. It was observed that there was some oil on the surface of the bath liquor, and also that the interior of the wash wheel was coated with a film of oil. The load was removed, hydro-extracted, and dried. Samples of the cotton were removed for oil and fatty acid analysis immediately after the souring operation and again after the oiling operation. The results of these analyses are given in Table III. The weight of oil deposited on the cotton in each oiling operation, expressed as a percentage of the weight of oil used, is also given.

The analyses of the residual bath liquors are misleading, owing to the fact that oil collected at the surface of the liquor in the wash wheel, and, on sampling, a high proportion of this oil was obtained. In the analysis of the residual baths from the first two loads, the samples were shaken vigorously to disperse the oil before removing aliquots for analysis. The sample from the third load was poured into a separatory funnel and allowed to stand for 10 min. before portions were drawn off for analysis. The figures obtained (Table III) are of no value as a criterion of the degree of exhaustion of the treating bath, but they do indicate that the coagulation of the emulsions has been virtually complete, and also that a certain percentage of the precipitated oil has not been picked up by the cotton.

TABLE III
ANALYSIS OF COTTON FOR FATTY ACID AND OIL CONTENT

Number of oilings	Samples removed prior to oiling		Samples removed after oiling		Oil deposition on cotton (% of oil used)	Oil content of residual bath (% of oil used)
	Fatty acid, %	Oil, %	Fatty acid, %	Oil, %		
1	0.36 0.27 0.30		0.32 0.46 0.33 0.35 0.40 0.37	3.0 5.0 3.3 3.7 5.3 3.8		34.8 28.2
	0.31		0.37	4.0	57.3	31.5
2	0.18 0.38 0.23 0.25 0.22 0.30	0.7 0.7 1.0 0.8 0.9 1.1	0.26 0.28 0.30 0.33 0.27 0.25	4.7 5.5 5.1 5.2 5.5 4.6		23.3 17.6
	0.26	0.9	0.28	5.1	60.0	20.4
3	0.25 0.29 0.30 0.23 0.25 0.23	1.0 1.4 1.4 1.5 0.8 0.9	0.37 0.35 0.29 0.32 0.29 0.26	6.2 5.3 6.0 5.7 6.7 5.0		0.8 1.3
	0.26	1.2	0.31	5.8	65.7	1.1

Discussion

In general the process appears to be satisfactory. There is no build-up of fatty acid on repeated oiling, and the build-up of oil is slight, since the residual oil content of the cotton is reduced to approximately 1% after laundering. The uniformity of oil distribution is quite satisfactory. There is, however, considerable loss of oil owing to deposition on the interior of the wash wheel, so that it is necessary to allow sufficient emulsion for 7% oiling in order to obtain 4 to 6% on the fabric. This film of oil was readily removed from the wash wheel by giving it one suds with soap and alkali followed by one or two rinses with water.

Satisfactory oiling has been accomplished with Type *B* emulsions. The positive emulsion is identical with that recommended for the oiling of wool (2), and the use of these emulsions would therefore be desirable in plants engaged in the oiling of both wool and cotton. On the other hand, these emulsions must be prepared and measured with considerable accuracy. The feasibility of doing this on a large scale can be determined only by actual plant trial. Increasing the proportion of emulsifying agent in both emulsions allows more latitude in handling, and this advantage must be balanced against the increased cost of materials.

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THE THERMAL INSULATION OF A DOUBLE PILE FABRIC AND ITS VARIATION WITH THICKNESS¹

P. LAROSE²

Abstract

Results of the measurement of the thermal insulation of a double pile fabric, the thickness of which was varied by compression, are given. Within the limits of the experiments the thermal insulation was found to vary linearly with thickness. The slope of the line corresponds to a value of 4.6 clo per in. thickness. The results are discussed in regard to those obtained by other investigators.

Introduction

The thermal insulation of fibrous materials, either in the loose state or as knitted or woven fabrics, has been measured by a number of investigators. In some cases attempts were made to derive a relation between thermal insulation and density by varying the density of the material (Finck (2)). In other tests the thickness of the material was varied while keeping the density constant (Babbitt (1)). Some of the investigators by using different materials varied thickness and density at the same time (Sale and Hedrick (6)) (Speakman and Chamberlain (7)). The present paper gives results of tests carried out on a fabric, the thickness of which was varied by compression. This procedure eliminates complicating factors arising from differences in construction when comparing different materials or fabrics. It also gives directly a relation between density and thickness, since the weight of material remains the same. Moreover, it has the advantage of giving directly the effect to be expected from the compression of such a fabric during wear.

The material tested was a double pile fabric of $\frac{5}{8}$ in. nominal thickness, such as that used for lining flying clothing worn by some of our aircrew during the war. The pile was made of 2/18's worsted yarn with 64's quality wool, and was woven on to a cotton ground fabric made with 2/20's yarn of warp and 1/8's in the weft. The number of ground ends per inch was 60, pile ends 20, picks per inch 48. The construction was such that the tufts were continuous from one side of the fabric to the other, in other words, the same tuft projected on both sides, so that the number of tufts per square inch, namely 160, was necessarily the same on either side. The total weight of the material was 23 oz. per sq. yd., of which 14 oz. was wool pile and 9 oz. ground fabric. The compressibility of this fabric as measured by the reduction of thickness when the pressure is increased from 0.1 lb. per sq. in. to 1.0 lb. per sq. in. was about 50%. The pressures required to compress the fabric to the thicknesses determined during the experiment were estimated from the known compressi-

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bility curve for this fabric. The pressures read from the curve are given in Table I. The range covered includes all the pressures to which such a fabric might be subjected in the course of normal wear. Kitching (unpublished work) has measured the pressures under an airman's clothing while the latter was in a sitting position and found them to be less than 1.0 lb. per sq. in. in most regions, although this figure was exceeded in some instances but was never more than 2.0 lb. per sq. in. For a man who is walking about and whose clothing is not being pressed against any object, the pressure is bound to be quite small.

Tests were also carried out on a similar fabric but made with a coarser wool (60's quality) in the pile, for the purpose of determining the effect of temperature. Since the tests were made for three different thicknesses of the material, the results, as well as those obtained with two samples of felt tested in the same way, are included for comparison.

Method

The thermal insulation of the fabric was determined with the apparatus described by Niven (5) for the measurement of fibreboard insulation. It is a typical hot plate apparatus used extensively to measure the thermal conductivity of homogeneous building materials. It is very similar to an earlier apparatus described by van Dusen (8). The hot plate is sandwiched between two samples of the material to be tested, which in turn are confined by two cold plates. Lateral heat flow is prevented by means of a guard ring. The hot plate and the cold plates are suspended from rails on which they are free to move. An arrangement is also provided by which the plates can be pressed against each other so as to compress the samples contained between them. The samples tested measured 18 by 18 in. including the area over the guard plate. In all the tests the cold plates were maintained at 33° F., and the temperature of the hot plate was adjusted to give a temperature difference of 26° to 30° F. except where otherwise indicated. The thickness of the sample was determined by measuring with a micrometer caliper the distance separating the plates at the four corners and taking the mean of the four readings. Before readings of temperature and heat input were taken, the apparatus was allowed to stand until equilibrium conditions were attained. Half a day was usually sufficient but in many cases the sample was allowed to stand overnight and readings were taken the next day. In order to average the effect of slight fluctuations, readings were taken every 15 min. for a period of two hours or more.

Results

The results of our experiments are summarized in Tables I, II, and III. Thickness was determined as described under "Method." The compression pressure was obtained from the compressibility curve, as mentioned in the introduction. The bulk density is readily calculated from the weight of the material per unit area and the thickness. The conductance is calculated

from the known conditions of the experiments, while the insulation is obtained from the conductance by conversion of units.

Δt in Table II represents the difference of temperature between the hot plate and the cold plates.

TABLE I

THERMAL INSULATION OF PILE FABRIC FOR VARIOUS DEGREES OF COMPRESSION.
PILE MADE FROM 64'S WOOL

Thickness, in.	Compression pressure, lb./in. ²	Bulk density, lb./ft. ³	Conductance, B.T.U./ft. ² /hr./° F.	Insulation	
				Clo	Clo/in. thickness
0.585	0.0	3.28	0.43 ₀	2.64	4.52
0.459	0.05	4.19	0.54 ₂	2.10	4.57
0.408	0.12	4.70	0.60 ₆	1.87	4.59
0.345	0.23	5.56	0.70 ₈	1.61	4.65
0.305	0.35	6.29	0.79 ₈	1.42	4.67
0.262	0.57	7.33	0.93 ₈	1.21	4.63
0.220	0.90	8.72	1.13 ₀	1.00	4.57
0.171	2.0	11.2	1.55 ₂	0.73	4.28

TABLE II

THERMAL INSULATION OF PILE FABRIC FOR VARIOUS DEGREES OF COMPRESSION AND FOR
VARIOUS MEAN TEMPERATURES. PILE MADE FROM 60'S WOOL

Thickness, in.	Bulk density, lb./ft. ³	Conductance, B.T.U./ft. ² /hr./° F.	Insulation, clo	Δt , °F.
0.537	3.53	0.48 ₇	2.33	36.0
0.428	4.42	0.60 ₄	1.88	38.5
0.428	4.42	0.59 ₉	1.90	45.4
0.428	4.42	0.59 ₈	1.90	52.3
0.428	4.42	0.59 ₇	1.91	59.1
0.428	4.42	0.60 ₄	1.88	93.4
0.193	9.80	1.35	0.84	47.1

TABLE III

THERMAL INSULATION OF TWO SAMPLES OF FELT

Thickness, in.	Bulk density, lb./ft. ³	Conductance, B.T.U./ft. ² /hr./° F.	Insulation, clo	Δt , °F.
0.147*	10.4	1.79	0.63	—
0.123*	12.4	2.05	0.56	—
0.451**	9.15	0.60	1.90	44.8

* White and high quality felt from fine wool.

** Coarse punched felt on hessian base and with coarse hair.

Since we are dealing with clothing material we have chosen to express the insulation in 'clo' units. The clo unit has been defined as "the amount of insulation necessary to maintain in comfort a sitting-resting subject in a normally ventilated room (air movement 20 ft./min. or 10 cm./sec.) at a temperature of 70° F. (21° C.) and a humidity of the air which is less than 50 per cent." (3).

The clo unit is equal to $0.18 \frac{^{\circ}\text{C.}}{\text{Cal./hr./sq. m.}}$

or $0.88 \frac{^{\circ}\text{F.}}{\text{B.T.U./hr./sq. ft.}}$

The above results for thermal insulation have been plotted against thickness in Fig. 1.

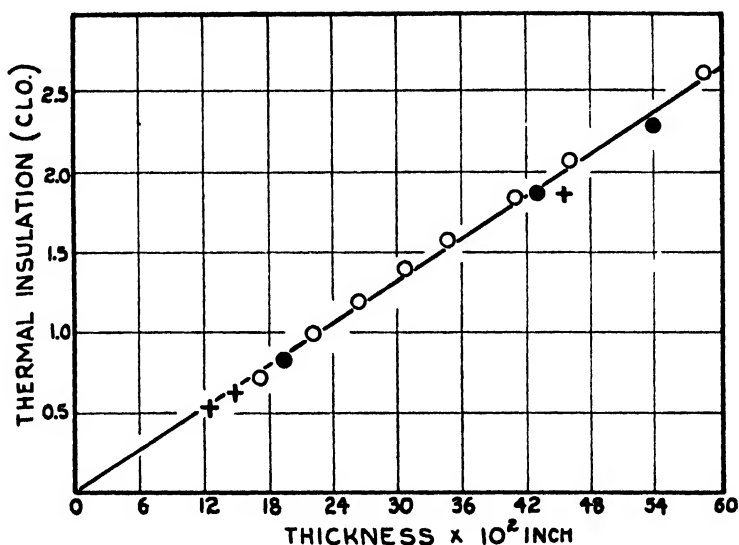


FIG. 1. Curve showing the relation between the thermal insulation of pile fabrics and thickness.

Discussion

Previous work carried out with fibrous materials has indicated that, within certain limits at least, the insulation varies linearly with thickness, but the relation was more or less obscured by complicating factors introduced by the use of materials that differed in some way or other when the thickness was varied. The work of Babbitt on fibreboard is an exception (1). He varied the thickness by removing material by abrasion, and not by compression, so that the density of the board was practically constant in all his experiments. His results for rock wool also apply to material with constant density. Nevertheless, his results are not comparable with those to be expected with a fabric under various degrees of compression. It is worthy of note that the insulation value per inch thickness was higher for the materials of lower density, e.g., the fibreboard of 12.2 lb. per cu. ft. gave an insulation (per inch thickness)

of 3.15 clo as against 2.65 clo for the board of 17.8 lb. per cu. ft. The rock wool gave a much better insulation, 4.25 clo for density of 7.9 lb. per cu. ft. and 4.83 clo for 7.1 density, but these two results are not strictly comparable, since the rock wool was not in the same form in the two experiments. Except for a surface effect in the fibreboard, Babbitt's results when plotted against thickness give practically a straight line passing through the origin. This shows that insulation was proportional to thickness within the limits of his experiments.

Marsh (4) obtained his results by testing materials that differed widely not only in thickness but also in density. He concluded that there was no relation between insulation value and density, but that there was a rough linear relation between insulation value and thickness. However, his curve did not pass through the origin. This he attributed to convection effects. His points are scattered widely about the curve, particularly for materials of low thickness. It should be noted that Marsh used a method in which the sample tested was wrapped around a cylinder and had therefore one of its faces exposed to the ambient air. Moreover, the thickness was taken as that under a certain arbitrary pressure. As his results are not expressed in terms that permit the calculation of the absolute conductivity of his materials, it is impossible to derive any relation from them.

The results given by Sale and Hedrick (6) were, like those of Marsh, obtained with widely different materials varying in thickness and density, and under such conditions that the materials were not completely enclosed, but had one surface exposed to the air. Nevertheless, a much better curve was obtained when their results were plotted. They did not show such a pronounced scatter as Marsh's results. Their curve also gave a zero intercept, which was explained by a surface effect. The best curve through Sale and Hedrick's results gives a slope of 2.93 clo per in., but, if the points for thicknesses less than 0.16 in. are omitted, a fairly satisfactory curve passing through the origin can be drawn. This curve has a slope of 3.91 clo per in.

Finck (2) measured the insulation of various materials at constant thickness but varied the density of packing. All his curves giving the relation between the insulation and density show a maximum (minimum when the points are plotted as conductance against density) for a particular density, which, for most materials, was around 5.0 lb. per cu. ft., kapok forming an exception with its maximum insulation at a density of about 1.0 lb. per cu. ft.

Speakman and Chamberlain (7) also found with some of the materials they investigated a decided change in the insulation as the density was varied. At the low densities their curves exhibit a maximum insulation (minimum conductivity) or a plateau for a narrow range of densities, but as the density is increased above a certain value (~ 10 lb. per cu. ft.) the insulation decreases at first slowly, then more rapidly.

Fig. 1, in which are plotted our results, shows distinctly that a straight line relation was obtained for the insulation in respect to thickness for all densities in the range of 3.2 lb. to 11.2 lb. per cu. ft. A close examination of the

insulation values per inch thickness in Table I reveals, however, a tendency for the insulation to increase and then decrease, with a maximum at a density of about 6 lb. per cu. ft., but, in view of the slight differences noted and the possible experimental error, the straight line was considered to represent the trend with sufficient accuracy.

It should be pointed out that in spite of the range of densities covered in our experiments, a range representing that which might be met in practice with such fabrics, the variation in this respect was not as large as that observed in the experiments of van Dusen or of Finck. Our results for the insulation of the double pile fabric at the lowest density and at a density of 8.7 lb. per cu. ft. differ by only 3.3 and 2.2%, respectively, from the highest value obtained at a density of 6.3 lb. per cu. ft. These differences are considered to be of the same order as the experimental error. The result for the highest density of 11.2 lb. per cu. ft. is the only one that shows a difference, $8\frac{1}{2}\%$, that may be significant. Finck's published results do not include wool but his results for the insulation of curled hair show a difference of 8% when the density was varied from 3.6 to 4.6 lb. per cu. ft. In the case of jute, a difference of 15% was obtained in changing the density from 3.5 to 12.3 lb. per cu. ft. Van Dusen got a difference of 19% when the density of wool was varied from 2.5 to 6.3 lb. per cu. ft. These variations are appreciably larger than those obtained in our experiments. Speakman and Chamberlain on the other hand give results that are comparable with ours. A 56's Australian crossbred wool showed a difference of 6.3% in insulation value as its density was changed from 0.8 to 11.0 lb. per cu. ft., but an 80's Australian merino wool showed hardly any variation in insulation as the density ranged between 1.5 and 10.1 lb. per cu. ft. while a 64's merino wool gave a difference of insulation of 2.9% within the same range of densities. It would seem therefore that for low densities, say up to 10 lb. per cu. ft., the insulation value of loose wool changes very little with density and is a function of thickness only.

Examination of Fig. 1 shows that the line drawn through the points representing the results for Table I passes through the origin. This is in agreement with the results of Babbitt already quoted and those of Sale and Hedrick, with the limitations cited above. The results of Speakman and Chamberlain also give straight lines, the slopes of which depend on the densities of the fabrics. The slope of our curve gives an insulation value of 4.60 clo per in., while the results of Table II give a mean insulation of about 4.4 clo per inch. For the purpose of comparison, some of the values obtained by the investigators referred to above are summarized in Table IV.

The above figures indicate that our results correspond more closely to those for loose materials than to the results for fabrics, which are definitely lower. The lower results for fabrics may be attributed to the higher density of the material. The density effect is intensified by the fact that the fibres are not uniformly distributed, and the actual density in the yarn is much higher than the bulk density calculated from the weight of the material and the apparent volume of the fabric.

TABLE IV

THERMAL INSULATION FOUND BY VARIOUS INVESTIGATORS FOR CERTAIN FIBROUS MATERIALS

Investigator	Material	Density, lb./cu. ft.	Insulation, clo/in. thickness
Finck	Kapok	1 0	4.75
Finck	Jute	3 5	4.56
Finck	Sphagnum moss	5 4	4.80
Van Dusen	Wool	6 6	4.65
Babbitt	Rock wool	7 1	4.83
Babbitt	Rock wool	7 9	4.25
Sale and Hedrick	Wool blankets	3.9 - 7 8	3 77
Speakman and Chamberlain	Wool fabrics	15.5 - 26	3 70 - 3 98
Speakman and Chamberlain	Loose wool (64's)	1 5	5 0
Speakman and Chamberlain	Loose wool (64's)	11 0	4.8
Speakman and Chamberlain	Milled fabrics	17 - 18	4.15

A straight line passing through the origin such as we obtained indicates that, even at the lowest density employed, the air is sufficiently broken up by the fibrous mass to render convection effects negligible. At the lowest density reached in our experiments, the fibres are separated by a mean distance of $\frac{1}{8}$ mm., a distance much too small for convection effects to have any importance. By comparing the thermal transmission of kapok layers placed firstly in a vertical position, then in a horizontal position, Finck showed that convection plays a negligible part in heat transmission along a fibrous mass. He concluded therefrom that radiation must be responsible for the changes noted at low densities. On the other hand, at high densities the radiation loss becomes quite small and conduction along the fibres must play a role in accounting for the increase in heat transmission.

Where a straight line relation holds, as in our experiment, it follows from the previous remarks that any increase in heat transfer due to conduction by the fibres must be accompanied by a corresponding decrease in radiation. Increased conduction along the fibres will take place as the density of the mass increases, for the proportion of the space occupied by the fibres increases. At the same time, transfer of heat by radiation must decrease since the path from hot to cold plates becomes more and more broken up by intervening fibres. This follows from the well known principle that intervening walls between radiator and receptor will reduce the amount of radiation owing to the fact that radiation then occurs between bodies with smaller temperature differences. This applies when the temperatures of the intervening surfaces are intermediate between those of the radiator and receptor, conditions that hold in the present instance. However, the increase in conduction through the fibres with increase in density is the result of several effects. In the first place, the increase of fibre substance by itself will increase conduction, as already mentioned, owing to the fact that the heat conductivity of the fibre

is greater than that of air. Secondly, the increase in density will no doubt result in a better contact between fibres and between the fibrous mass and the hot and cold walls. Both these effects have been mentioned by Finck in discussing the results of his experiments. There is a third factor, however, in connection with conduction by the fibres which does not seem to have been considered by previous investigators, namely, the length of the fibres in relation to the thickness of the insulating layer. Finck showed that the direction of the fibres had quite an effect on the value of the insulation, the insulation when the fibres lay perpendicular to the direction of heat flow being in some cases more than double that obtained when they were parallel to the direction of flow. This effect is an important one because it means that for any one type of fibre and given density, a wide range of values, depending on the orientation of the fibres, can be obtained for the insulation. Finck correctly interpreted the effect but failed to mention the fact that this effect could be influenced considerably by the length of the fibres. The short circuiting of the plates by the fibres when these are parallel to the direction of heat flow can occur only if the fibres are longer than the distance separating the plates, otherwise there must be a break in the conduction along the fibres, and heat transfer has to take place through the contact points between fibres. Naturally, the shorter the fibres, the greater the number of such breaks and the greater the resistance to heat flow along the fibres. This effect has probably been disregarded because, in most experiments, the thickness of the layer tested was small or of the same order of magnitude as the length of the fibre. In the case of wool certainly this must be so. Our pile fabric was so constructed that the fibres in the wool tufts constituting the pile were continuous from one side to the other, and in all tests short circuited the hot and cold plates of the apparatus. However, the pile is so cut that the fibres are not all of the same length. At low densities therefore, when there is little pressure applied to the fabric, the contact with the plates would be a poor one, but, as the pressure is increased, not only do more and more fibres make contact with the plates but the contact also becomes closer in that the fibres are more firmly pressed against the plates. It must be pointed out, however, that the pile as a whole does not lie parallel to the direction of heat flow, but makes a small angle with it. Moreover, the wool fibres are not straight but are crimped. Parts of the fibres will therefore lie in various directions quite different from the general direction of the pile.

As the pile is compressed the fibres will be distorted, or they will be bent over. In any case the compression will cause the direction of the fibres to become more and more nearly perpendicular to the direction of heat flow, and this will tend to reduce the transfer of heat by radiation, as was pointed out by Finck. On the other hand, the better contact with the plates that results tends to increase conduction, although the actual path along the fibres does not necessarily become shorter. It would appear from our results that these two opposing effects just about balanced each other.

Acknowledgment

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THE PREPARATION OF HEXACHLOROETHANE IN THE LIQUID PHASE BY CHLORINATION OF TETRA— AND PENTACHLOROETHANE¹

BY JESSE A. PEARCE²

Abstract

Preliminary investigations showed a slow rate of production of hexachloroethane from chlorine-saturated liquid tetrachloroethane. The addition of some materials that often accelerate similar reactions was not effective here. However, rapid production was obtained by chlorinating tetrachloroethane in the presence of ultra-violet light. The effective wave-lengths appeared to lie between 3150 Å and 3540 Å, and the temperature coefficient between 75° and 100° C. was 1.10. The result indicated that production of hexachloroethane from chlorine-saturated liquid tetrachloroethane was feasible. For the same conditions of illumination and temperature hexachloroethane was produced from chlorine-saturated pentachloroethane at a rate two and one-half times as fast as that in chlorine-saturated tetrachloroethane.

Introduction

In the past the commercial preparation of hexachloroethane has involved preparation of trichloroethylene from tetrachloroethane, followed by chlorination to pentachloroethane, the decomposition of pentachloroethane into tetrachloroethylene, and chlorination of this last product to hexachloroethane.

Two other methods of preparing this product from tetrachloroethane are also known. One method, which depends on the chlorination of tetrachloroethane using ultra-violet light or other light source rich in active rays, probably yields a mixture of penta- and hexachloroethanes (7, 8). Whether this was done using liquid tetrachloroethane or the vapour is uncertain. The other method, involving chlorination of liquid tetrachloroethane in the presence of vegetable charcoal, animal charcoal, bleaching powder, ferric chloride, or aluminium chloride as catalysts, is reported to yield only hexachloroethane (3). The study reported in the present paper was made in 1940, when a large increase in Canadian production of hexachloroethane seemed necessary, and it was suggested by Dr. O. Maass that the possibility of manufacturing this material by direct chlorination of tetrachloroethane in the liquid phase should be investigated.

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Contribution from the Department of Chemistry, McGill University, Montreal, P.Q. Material selected from a thesis presented to the Faculty of Graduate Studies and Research, McGill University, in partial fulfilment of the requirements for the degree of Doctor of Philosophy.

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Thermal Studies

Materials

Initially an attempt was made to use crude tetrachloroethane as currently prepared on a commercial scale. However, this material was unsatisfactory and several methods of purification were attempted. The materials used are described in Table I.

TABLE I

EFFECT OF STARTING MATERIAL ON THE RATE OF FORMATION OF HEXACHLOROETHANE FROM TETRA- AND PENTACHLOROETHANE AT 100° C.

(Illumination variable)

No.	Starting material	Degrees of freedom	Average rate*	Standard deviation
I	Crude tetrachloroethane	—	Decomposition	—
II	Fraction of No. I boiling at 144 to 148° C.	2	3 8	0 36
III	No. II after drying over calcium chloride	4	2 2	0 71
IV	No. III partially chlorinated, then fractionated. Fraction boiling at 145 to 147° C. used.	1	6 4 (2 3, 10 6)	—
V	Tetrachloroethane; b p., 146 3° C.	5	2 8	2 1
VI	No. V after being subjected to some decomposition by heating with aluminium chloride, followed by fractionation. Fraction boiling at 144 to 148° C. used.	3	2 6	0.77
VII	Mixture; III : V = 2 : 1	7	1 5	0 77
VIII	Pentachloroethane; b p., 161 7° C.	1	3 2 (1 3, 5 0)	—

* Rates given in Tables I and III record the percentage of hexachloroethane formed in the reacting mixture per hour, averaged over chlorination periods of about 20 to 36 hr. Those given in Table IV are for chlorination periods of one to four hours.

Analytical Methods

An analytical method that proved satisfactory was based on the change in boiling point as the quantity of the hexachloroethane in solution was increased. Boiling point calibration curves for hexa- in tetrachloroethane and hexa- in pentachloroethane were established (Fig. 1). Several samples of partially chlorinated tetrachloroethane, when fractionated in a Whitmore column, gave yields of hexachloroethane that did not differ significantly from the amount predicted by boiling point determinations. Pentachloroethane, if present, was not detectable by these fractionations; this supported a previous similar observation (3).

Apparatus

The rate of production of hexachloroethane was readily followed by the boiling point technique in apparatus of the type shown in Fig. 2. Unless otherwise noted these were constructed of Pyrex. The portion designated *A* in Fig. 2 had a capacity of about 50 ml., while the enlarged part, *B*, helped

eliminate bumping of the material into the condenser, *C*, when the material was boiling. The chlorine inlet is shown at *D* and was connected directly to a chlorine cylinder by rubber tubing. Chlorine flow during a boiling point

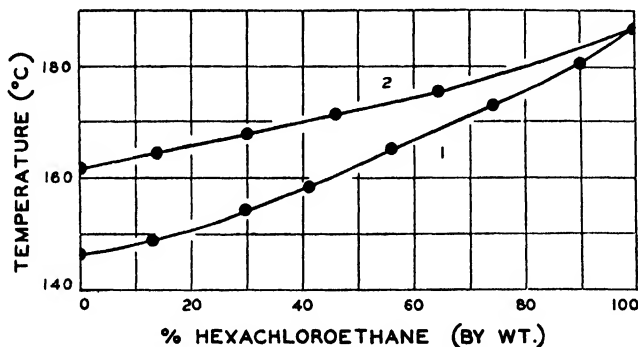


FIG. 1. Boiling-point-composition curves for solutions of hexa- in tetrachloroethane (Curve 1) and of hexa- in pentachloroethane (Curve 2.)

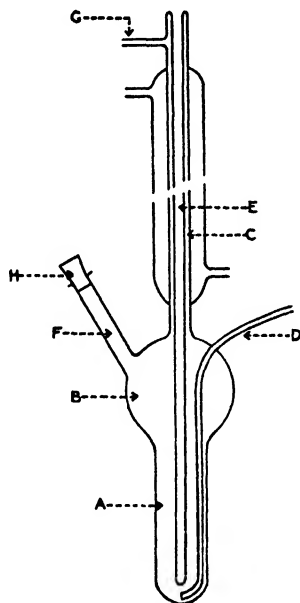


FIG. 2. General design of chlorination apparatus.

determination reduced the temperature and was, therefore, stopped with a screw-clamp. At the same time, this made *D* an enclosed space, which helped prevent superheating. In the diagram, *E* designates a thermometer well, which contained a clear, high-boiling oil. Tetrachloroethane and catalyst were introduced through the arm, *F*, which also facilitated cleaning. Excess chlorine was conducted away at *G*. Modifications of this apparatus are described in Table II.

Temperatures of the reaction mixtures were controlled by suitable oil- or water-baths, or direct flame. Boiling-point-composition curves and certain other boiling point determinations were made in an apparatus of similar design, but the portion *A* had a capacity of only 10 ml. and the other dimensions were correspondingly reduced.

TABLE II

EFFECT OF SOME FACTORS ON THE RATE OF FORMATION OF HEXACHLOROETHANE FROM TETRACHLOROETHANE AT 100° C.

(Illumination variable)

Factor	Average rate
<i>Surface area:</i> reaction chamber packed with Pyrex chips	2.2
<i>Surface quality:</i> reaction chamber made from quartz	2.7
<i>Liquid depth:</i> reaction chamber made 7 ft. in length	2.9
<i>Condenser volume:</i> bulb above liquid chamber made 7 ft. in length, i.e., reaction in gas phase above liquid.	2.9
<i>Pressure:</i> reaction at 1½ atm.	0.4

Chlorination Procedure

A weighed quantity of tetrachloroethane (usually 50 gm.) was introduced into the reaction chamber and saturated with chlorine, while being brought to the desired temperature. Constant introduction of chlorine during the reaction maintained the chlorine content of the mixture as nearly as possible at saturation.

Unless otherwise noted the reaction chambers were subjected to no other pressure than atmospheric. The duration of any reaction varied from 20 to 36 hr., the actual time depending on the conditions of the experiment and the rate of production of hexachloroethane.

Results

It was observed (Table I) that neither the purity of the material nor method of purification had any effect in improving the rate of hexachloroethane production provided the tetrachloroethane had received sufficient purification to prevent its decomposition. Attempts to purify the material by allowing it to stand over potassium hydroxide, followed by distillation, were unsatisfactory, since sufficient alkali was dissolved to cause decomposition during the distillation (9). The data in Table I also indicate that the rate of production from pentachloroethane was not appreciably different from the rate of production from tetrachloroethane.

Examination of the rates of production in different pieces of apparatus indicated that somewhat faster rates occurred in one reaction vessel. These faster reaction rates were attributed to several possible factors, e.g., increased surface to volume ratio of liquid in this chamber. These factors and their

effects on production rates were examined and the results are shown in Table II. Increase in pressure, the only factor having a significant effect on rate, appeared to retard the production of hexachloroethane.

The addition of substances ordinarily believed of value in accelerating chlorinations had no effect on the rate of this reaction (Table III). Many added substances caused charring in the reacting mixture, indicating decomposition of the tetrachloroethane; charring was generally accompanied by a

TABLE III

EFFECT OF ADDED SUBSTANCES ON THE RATE OF FORMATION OF HEXACHLOROETHANE FROM TETRACHLOROETHANE AT 100° C. AND AT THE BOILING POINT OF THE REACTING MIXTURE

(Illumination variable)

Added substance		Average rate	
		At 100° C.	At B.p.
Activated charcoal		Decomposition	—
Aluminium chloride	10.0%	—	Decomposition
	0.5%	—	2.2 (sl. dec.)
	0.2%	3.6 (sl. dec.)	2.7 (sl. dec.)
Ferric chloride	0.2 to 10%	Decomposition	Decomposition
Stannous chloride	2.0%	—	0.6 (sl. dec.)
	0.2%	—	1.2
Nickelous chloride	2.0%	—	0.5
Sulphur monochloride	20%	—	1.1
	2%	—	1.2
	0.2%	—	0.4
Sulphuryl chloride		3.1	—
Benzoyl peroxide		2.9	—
Saturation with water		2.9	—
Nickel gauze		3.2	—

decrease in boiling point. Since dechlorination of the tetrachloroethane vapour is known to occur over charcoal at 400° C. (11), it was suspected that charcoal caused decomposition in the liquid phase at 100° C. Nickel gauze was added to ascertain whether serious corrosion occurred, or whether the reaction rate might be affected, i.e., whether nickel could be used as a reaction vessel. There was practically no corrosion and no change in rate.

It is also evident from Table III that production at 100° C. was preferable to production at the boiling point, while at 25° C. no reaction occurred.

Photochlorination Studies

Since none of the factors studied had any appreciable effect on the rate of thermal chlorination, some attention was given to the photochemical reaction in the liquid phase. The preliminary results (Table IV and Fig. 3) indicated that light was effective in accelerating the reaction, that the ratio of surface

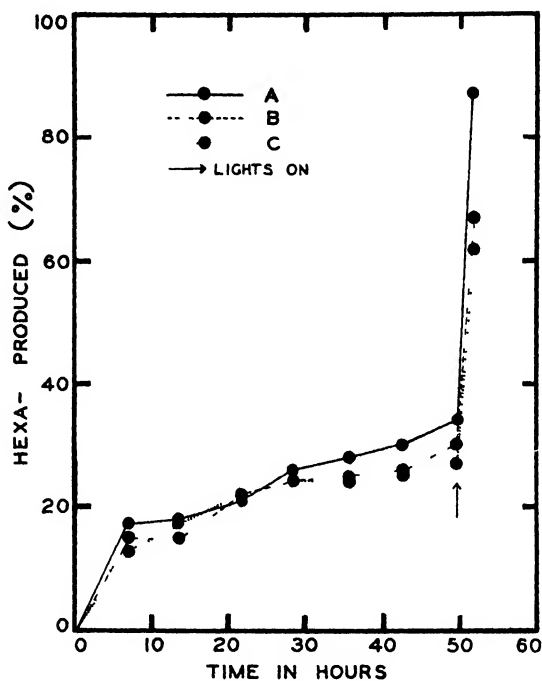


FIG 3 Production of hexachloroethane at 100° C from chlorine saturated tetrachloroethane in the dark and during exposure to seven 500 w lamps (A, B, and C designate different pieces of apparatus, A had a slightly larger surface to volume ratio)

to volume of the reacting liquid may have effect on the rate, and that 100° C. was the preferable temperature for the reaction. Further work was confined, therefore, to an evaluation of the photochemical production of hexachloroethane from chlorine-saturated liquid tetrachloroethane.

Materials and Analytical Methods

Initially a fraction of crude tetrachloroethane boiling at 144° to 148° C. was used. However, as a result of anomalous rates (Fig. 4,A) with this material, the pure material boiling at 146.3° C. was used for further work. The pentachloroethane used was similar to that described in the thermal studies.

The rate of production of hexachloroethane was again determined by measurements of the boiling point of the reacting mixture in an apparatus of the type previously described.

TABLE IV

EFFECT OF LIGHT AND TEMPERATURE ON THE RATE OF FORMATION OF
HEXACHLOROETHANE FROM TETRACHLOROETHANE

Lighting condition	Temperature, °C.	Degrees of freedom	Average rate	Standard deviation
Morning (sunlight)	100	38	3.9	1.9
Afternoon (diffused daylight)	100	26	0.9	0.91
Night (without artificial illumination)	100	11	0.6	0.47
Light-proof chamber	100	17	0.8	0.94
Morning (sunlight)	100	8	2.5	0.63
One 500 w. incandescent lamp	100	4	18.3	5.2
Seven 500 w. incandescent lamps	43	1	19.3	—
Seven 500 w. incandescent lamps	100	4	22.2	4.0
Seven 500 w. incandescent lamps	B.p.*	2	12.7	8.1
Seven 500 w. incandescent lamps	B.p.†	2	4.4	3.3

* Heating by open flame.

† Heating in oil-bath.

Apparatus

The photochlorination cell was essentially similar to that shown in Fig. 2 except that the reaction chamber consisted of a Pyrex flask 8 cm. in diameter with a flat Corex glass bottom, the top being drawn down for attachment to the condenser and chlorine inlet. All portions of the apparatus except the bottom plate were painted black to prevent the entry of stray light. The reaction chamber was enclosed in an air cabinet regulated to the desired temperature (75° or 100° C.) and the bottom of the chamber was approximately seven inches from the centre of a Mazda incandescent lamp (200 or 500 watts). These lamps emitted rays from 2850Å to beyond 6000Å, were operated slightly below 110 v., without a filter between the lamp and the reaction vessel, and were maintained at a constant intensity by a Corning filter transmitting 3300 to 3900Å, a photocell, a galvanometer, and a variable resistance.

Chlorination Procedure

This was essentially similar to that previously described, the only difference being exposure of the reaction chamber to constant light sources and variations in depth of the reacting mixture.

Results

(1) The Light Absorbed

A spectrograph was used in an attempt to determine the wave-lengths absorbed by the reacting mixture, i.e., the wave-lengths that may have been effective in accelerating the reaction. Tetra- and pentachloroethane, and a 50-50 solution of hexa- in tetrachloroethane were all transparent to light in

the region 3150 to 6000Å, but saturation of these solutions with chlorine rendered them opaque to wave-lengths from 3150 to 4600Å. This roughly corresponds to the absorption range observed for chlorine gas (1). It has been reported that di-, tri-, tetra-, penta- and hexachloroethane are transparent to mercury lines 3650Å and 4360Å, but that the presence of chlorine renders these solutions opaque to these wave bands (2).

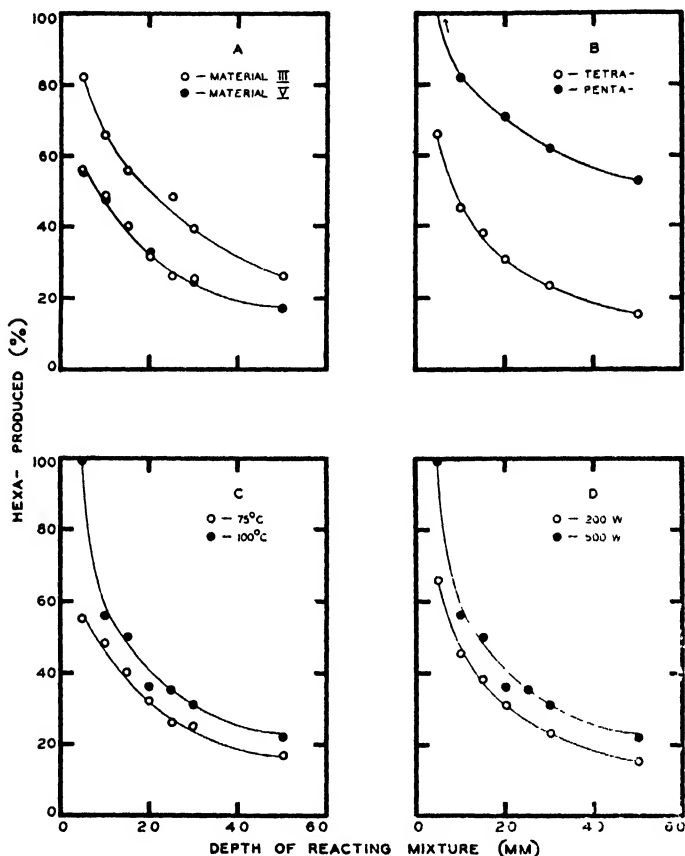


FIG. 4. The effect of depth of chlorine-saturated tetrachloroethane and other factors on the rate of production of hexachloroethane.

- A. Exceptional rates with less pure material (III) shown in comparison with rates for more highly purified material (V).
 B. Production of hexa- from penta- and tetrachloroethane at 100° C. using a 200 w. lamp.
 C. Production of hexa- from tetrachloroethane at 75° and 100° C. using a 500 w. lamp.
 D. Production of hexa- from tetrachloroethane at 100° C. using a 500 and a 200 w. lamp.

(2) The Effect of Purity of the Starting Material

Rate determinations were made using different volumes of tetrachloroethane in the reaction flask. The results are given as the per cent hexachloroethane in the reacting mixture after 30-min. periods for the various depths of the reacting mixture used (with 0.5 ml. layers only a 15 min. reaction

period could be used, but the results have been calculated to a 30 min. basis). The first rate measurements with tetrachloroethane boiling at 144° to 148° C. showed this material to have variable reaction rates (Fig. 4,A). Determinations with material of high purity indicated that a slow rate was typical of hexachloroethane production in the absence of impurity.

(3) *Comparison of Rates Using Penta- and Tetrachloroethane*

While no pentachloroethane was found when fractionating partially chlorinated samples of tetrachloroethane, it was possible that the chlorination was a two stage reaction, the tetra- first going to penta- and the penta- then forming hexachloroethane at a slower rate. However, chlorinations using these two materials showed that hexachloroethane was formed from penta- about two and one-half times as fast as from tetrachloroethane (Fig. 4, B). This indicated that, if a two stage reaction were occurring, the second stage had little significance in determining reaction rates.

(4) *Effect of Temperature*

The curves in Fig. 4, C, show the effect of temperatures of 75° and 100° C. on the photochlorination. It is evident that an increase in temperature in this range caused an increase in the rate of production of hexachloroethane. Generally about 25% more material was produced at the higher temperature in the same reaction time. When calculating a temperature coefficient for practical use (1.10), differences in the solubility of chlorine in tetrachloroethane at the two temperatures were neglected.* This value is of the same order as the value of 1.16 reported for chlorination, by addition, of tetrachloroethylene (4), of 1.13 for chlorination of dichloroethylene to tetrachloroethane (5, 6), of 1.21 for the chlorination of trichloroethylene to pentachloroethane (6), and 1.12 for the bromination of tetrachloroethylene (10).

(5) *Effect of Light Intensity*

The effect of light intensity is shown in Fig. 4, D. The reaction rate in the presence of the 500 w. lamp was only 1.3 times that with a 200 w. lamp. The ultra-violet intensity in the region 3300 to 3900Å from the 500 w. lamp was found to be 1.7 times that from the 200 w. lamp. Since the square root of this intensity ratio was the same (1.3) as the rate ratios, it was believed that the reaction rate varied as the square root of the incident light intensity. A similar observation had been made previously for the chlorination of tetrachloroethylene (4).

(6) *Effect of Depth of Reacting Mixture*

The curves shown in Fig. 4 clearly depict the pronounced decrease in reaction rate that resulted from increase in depth of the reacting mixture. From the results shown, calculations have been made to indicate the produc-

* A solubility value at 100° C., of 0.16 moles of chlorine per litre of tetrachloroethane had been determined. This was verified by data kindly supplied by the Electrochemical Department of the E. I. du Pont de Nemours and Co., Inc. From the data supplied, a solubility, at 75° C., of 0.27 moles per litre was calculated by interpolation.

tion occurring in each $\frac{1}{2}$ -mm. layer, assuming that no mixing occurred between layers of the liquid. The results of these calculations (Fig. 5) show that, regardless of the temperature and light intensity used, the rate of conversion became negligible when the depth of liquid exceeded 1 mm. The decrease in rate between the first and second $\frac{1}{2}$ -mm. layers indicated that only 20% of the most effective light passed the first $\frac{1}{2}$ mm. of the reacting mixture. The

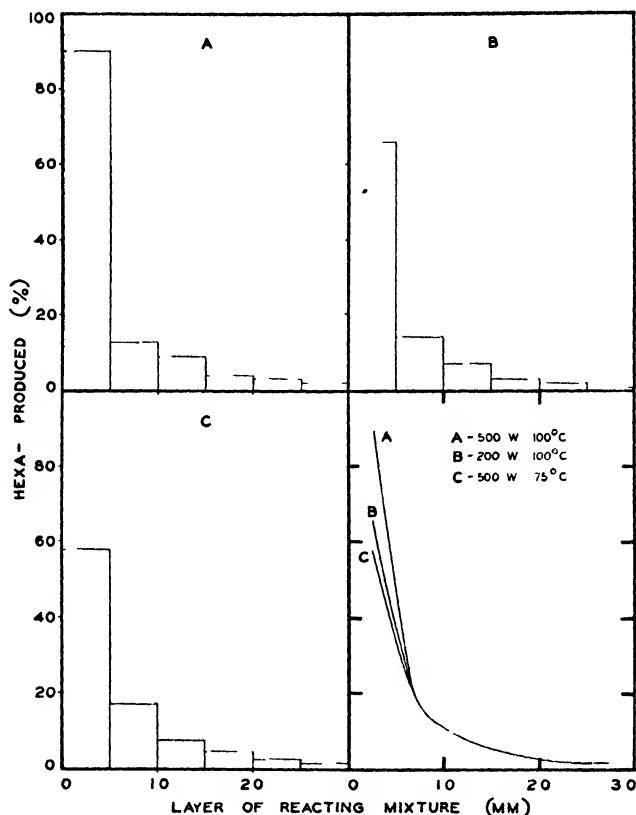


FIG. 5. Production of hexa- in respective layers of chlorine-saturated tetrachloroethane (calculated from values shown in Fig. 4) at temperature and lighting conditions noted.

less pronounced decrease in rate in the succeeding layers may result from a slight activity of the less highly absorbed wave-lengths of the activating light or from agitation of the liquid by the incoming gas. It is of little importance, however, since less than 10% of the product was formed in these layers.

Calculations utilizing a solubility value at 100° C. of 0.16 moles of chlorine per litre of tetrachloroethane, the foregoing evidence, and the extinction data for chlorine gas (1) indicated that rays between 3150Å and 3540Å were most effective in accelerating this substitution reaction. This is somewhat lower than the wave-length used for addition reactions (4, 5, 6, 10).

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REDUCTION OF SPATIAL TEMPERATURE VARIATIONS IN AIR-COOLED STORAGE ROOMS. II.¹

By J. W. HOPKINS², T. A. STEEVES³, AND W. H. COOK⁴

Abstract

Measurements made under a series of imposed conditions of heat load, dunnage spacing, and air flow were in agreement with previous work in this laboratory in demonstrating (a) the occurrence of appreciable permanent temperature gradients in material, whether exothermic or not, stacked in an air-cooled storage room, and (b) the possibility of markedly reducing these gradients by effective channelling of air through the stack. With end-to-end circulation, blocking of voids in the room was the most important single factor in minimizing intra-stack temperature differentials under the conditions of these tests. Further improvement was effected by the provision of optimum dunnage and by augmenting the air flow. It is to be inferred that with blocked voids, dunnage should be extended to all external surfaces of the stack. The desirability of uniform transverse and vertical distribution of the circulating air was also evident. Further trials on a larger scale are required to explore the practical implications of these findings.

Introduction

A previous communication from these laboratories (4) described measurements of spatial temperature variations in an experimental storage room provided with bottom-to-top air circulation. Tests were made first in the empty room, then after introducing a false load of empty boxes to obstruct the air flow, and finally after the addition of known stack heat loads generated by electrical elements placed centrally in the first layer of boxes. Presence of the boxes, even without heat, was found to increase significantly the spatial variation in temperature. Addition of heat, as expected, accentuated this effect. It was found that blocking all voids surrounding the stack itself led to a significant reduction in the spatial temperature differences within the stack. Even more pronounced reductions (of the order of 60%) resulted from the provision of dunnage spacing between each horizontal layer and also between each pair of vertical piles of boxes.

It is now desired to report upon a further and more extensive series of observations made in a somewhat larger experimental storage room. In these trials the preceding bottom-to-top air flow was replaced by an end-to-end circulation more characteristic of large warehouses, and the experiments comprised various stack heat loads, dunnage spacings, and air flows. The results demonstrate that, under the conditions specified, blocking of the voids above and at the side was by far the most important single factor in reducing

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the accumulation of heat within the stack. Further reductions resulted from dunnage spacing (which was optimum in the vicinity of $\frac{3}{8}$ to $\frac{1}{2}$ in.) and from increased air flow. It is also shown that a major part of the spatial temperature differences recorded within the stack took the form of systematic gradients, transverse and vertical as well as longitudinal, and some of the features of these characteristic of various operating conditions are investigated analytically by a series of plane sections of isothermal surfaces.

Experimental

The experimental room, which was of about 1600 cu. ft. capacity, measured internally 16 ft. in length, 13 ft. in width, and 8 ft. in height. It was insulated by a 4 in. layer of cork. Wooden boxes of size 1 by 1 by 2 ft. were stacked in the room 12 long, 5 wide, and 6 high, giving a stack of dimensions 12 by 10 by 6 ft. exclusive of dunnage. Dunnage (spacing strips) was applied in both the horizontal and vertical plane to each vertical group of six boxes individually, and was also applied externally to the top, bottom, and sides of the stack. A free space of rather more than 3 ft. at the front end of the stack accommodated an exhaust blower refrigerator coil centrally located and discharging through a sheet metal duct past one side of the stack into a smaller free space, rather less than 1 ft. across, between the back of the stack and the rear wall of the room. One side of the stack was positioned $1\frac{1}{2}$ in. plus dunnage from the side wall of the room. Insulating board placed 1 in. plus dunnage from the opposite side of the stack created a void roughly 3 ft. across between this and the opposite wall. Through this void, which was obstructed by baffles at the front and rear (but not at the top) of the stack in order to prevent "short circuiting" of the air circulation, passed the above-mentioned air duct. The combined effect of these features was to leave free spaces of approximately 3 ft. at the front, 2 ft. at the top, 1 ft. at the back, and $1\frac{1}{2}$ to 1 in. plus dunnage at the two sides of the stack. A spacing similar to the last, namely, 1 in. plus dunnage, was also maintained between the bottom of the stack and the floor. All boxes in the stack were equipped with electrical heating elements connected in five parallel series along the length of the stack and so installed as to ensure the generation of equal amounts of heat in all boxes at all dunnage spacings. Thermocouples were installed at each of 23 positions in the stack as specified in Table I, and also in the exhaust and discharge air ducts. The complete experimental arrangement is shown schematically in Fig. 1.

Two series of observations were made. For the first the system was operated at stack heat loads of 0, 432, and 864 B.t.u. per hr. in conjunction with air flows of 500, 710, and 920 c.f.m., dunnage widths of $\frac{1}{8}$, $\frac{3}{8}$, and $\frac{1}{2}$ in., and with the voids above and at the side of the stack both open and blocked. For the second the voids above and beside the stack were blocked throughout but air flows of 290, 500, 710, 920, and 1130 c.f.m. were employed in conjunction with stack heat loads of 0, 432, and 864 B.t.u. per hr. as before, and with dunnage widths of 0, $\frac{1}{4}$, and $\frac{1}{2}$ in. The variable stack heat loads specified were of course additional to the more or less constant amounts entering from

without through the walls and generated inside the room by the fan motor. Calculations based on recorded differences between inlet and exhaust air temperatures indicated that these latter, which may perhaps be termed the

TABLE I
CO-ORDINATES OF LOCATIONS OF THERMOCOUPLES IN STACK, RELATIVE TO
POINT SHOWN AS BOTTOM LEFT-HAND CORNER IN FIG. 1 (PLAN)

Thermocouple No.	Co-ordinates, ft.		
	Longitudinal	Transverse	Vertical
1	1.0	1.0	5.0
2	1.0	9.0	5.0
3	1.0	5.0	3.0
4	1.0	1.0	1.0
5	1.0	9.0	1.0
6	3.0	5.0	4.5
7	3.0	2.5	3.0
8	3.0	7.5	3.0
9	3.0	5.0	1.5
10	6.0	1.0	5.0
11	6.0	9.0	5.0
12	6.0	5.0	3.0
13	6.0	1.0	1.0
14	6.0	9.0	1.0
15	9.0	5.0	4.5
16	9.0	2.5	3.0
17	9.0	7.5	3.0
18	9.0	5.0	1.5
19	11.0	1.0	5.0
20	11.0	9.0	5.0
21	11.0	5.0	3.0
22	11.0	1.0	1.0
23	11.0	9.0	1.0

"maintenance heat load," averaged about 1600 B.t.u. per hr. with the voids open and about 1900 B.t.u. per hr. with the voids blocked, the difference of 300 B.t.u. per hr. presumably representing the extra mechanical work required to maintain the same air circulation against increased resistance. The maximum stack heat load was thus of the order of 50% of the maintenance load.

Blocking of the voids was effected as in the earlier experiments (4) by the inflation of suitably sized latex-coated shelter duck bags. Insulating board was interposed to maintain a space of 1 in. plus dunnage between these and the top of the stack. Ideally therefore air should have flowed with equal freedom over the top, bottom, and internal side surfaces. Actually, circulation was to some extent impeded above the stack by heater connections, and below it by thermocouple leads. With the voids blocked, therefore, the four surfaces in question were classified in the following order of increasing impedance to air flow: (i) outer side (adjacent to wall); (ii) inner side (adjacent to insulating board); (iii) top; (iv) bottom.

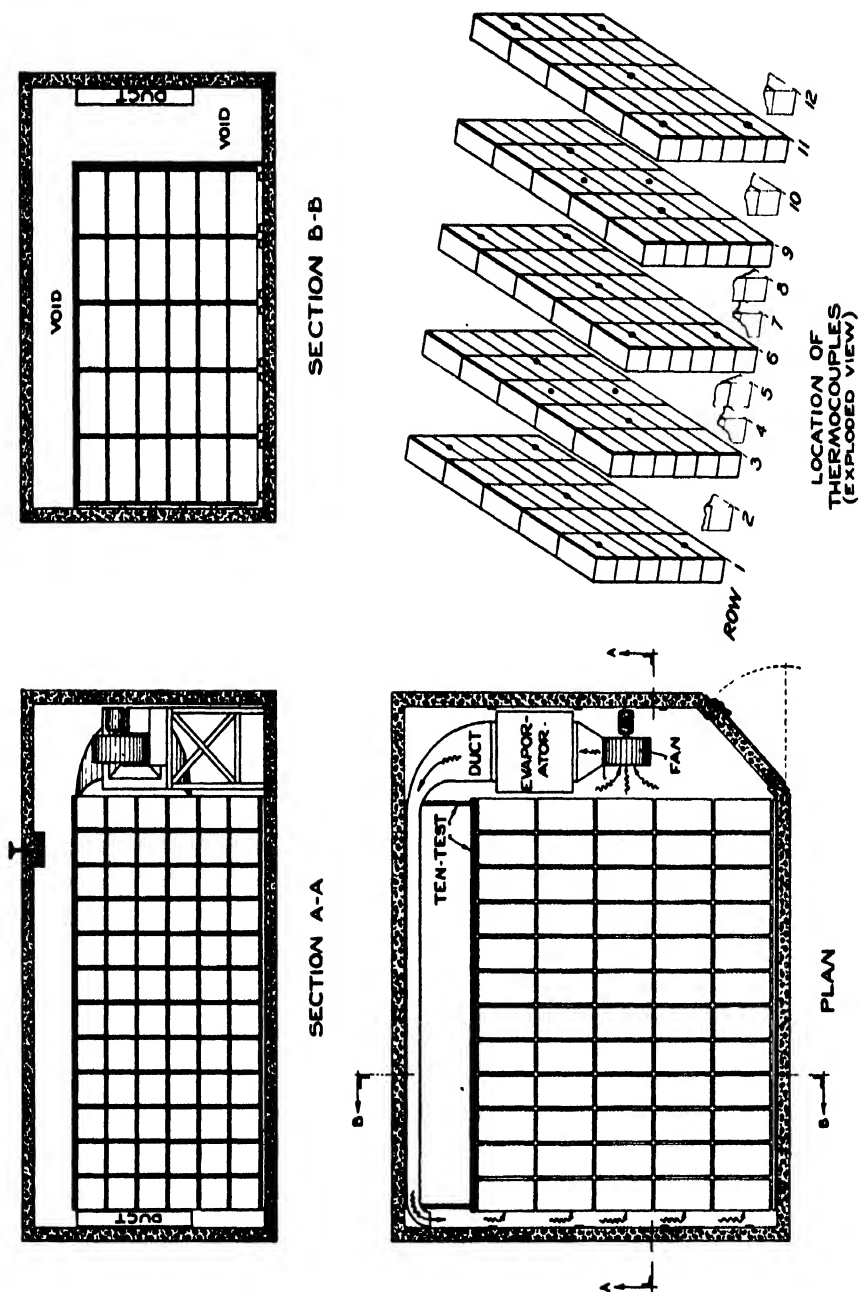


FIG. 1. Schematic diagram of test room and stack of boxes.

After the imposition of any selected set of conditions the system was operated for several hours (usually overnight) until equilibrium was attained. Ten readings of each thermocouple were then made at 20-min. intervals and averaged to yield a mean value characteristic of the position in question. Analysis of the entire series of observations thus involved the statistical reduction of some 23,000 individual items.

Results

Assessment of Significance

Although every effort was made to operate under controlled conditions of temperature, air flow, and heat load, for a variety of reasons this aim proved to be impossible of complete achievement, and the precise temperature gradients within the stack characteristic of any particular set of imposed conditions were never completely reproducible. The residual uncontrolled variation although generally small and of random incidence was statistically significant, and in these circumstances it was necessary to have recourse to the theory of errors for assessment of the data. The method adopted was to subject any function of the observations selected for study to an analysis of variance procedure (1, 2, 3) in which second-order interactions and differential effects, including the above-mentioned uncontrolled residual fluctuations, were used to test the statistical significance of differences in the averages of the main factors or their first-order interactions. The findings may conveniently be considered under the three headings of (a) total spatial temperature variance within the stack, (b) the proportion of this occurring in the form of systematic temperature gradients, and (c) the characteristics of such gradients.

Total Spatial Temperature Variation

The standard, i.e., root-mean-square, deviation of the average reading of each of the 23 thermocouples located within the stack was used as a measure of over-all spatial variation in temperature. This provides a satisfactory index of the relative variability encountered under different conditions imposed. It requires to be noted, however, that as a major portion of the variation in question proved to be systematic, the absolute values obtained in this way are applicable only to the particular ordered arrangement of the thermocouples adopted in these tests.

The main trends observable are summarized in Tables II to IV and illustrated in Fig. 2, A-D. Table II and Fig. 2, A-B, indicate that blocking the upper and side voids, thus forcing more of the circulated air to pass through the stack, markedly reduced the spatial variation at all three stack heat loads, including zero. It is to be noted, however, that whereas with the voids blocked minimum variation was recorded at $\frac{3}{8}$ in. dunnage, with the voids open the widest spacing used ($\frac{3}{4}$ in.) gave the least variation, suggesting that in this circumstance still wider spacing might have been advantageous. Table III and Fig. 2, C, obtained by combining relevant portions of both the first and second series of experiments, permit a comparison of six dunnage

TABLE II

INDEX OF SPATIAL TEMPERATURE VARIATION (STANDARD DEVIATION IN DEG. F.) FOR VARIOUS CONDITIONS OF DUNNAGE, AIR FLOW, AND HEAT LOAD

Dunnage, in.	Air flow, c.f.m.	Voids open				Voids blocked			
		Stack heat load				Stack heat load			
		0	432 B.t.u./hr.	864 B.t.u./hr.	Average	0	432 B.t.u./hr.	864 B.t.u./hr.	Average
$\frac{1}{8}$	500	2.3	2.9	3.7	2.95	0.7	1.5	2.2	1.47
	710	2.4	2.9	3.2	2.80	0.9	1.8	2.5	1.76
	920	2.0	3.1	3.6	2.89	0.4	1.8	2.4	1.52
	Average	2.23	2.93	3.44	2.87	0.67	1.71	2.36	1.59
$\frac{1}{4}$	500	2.6	3.1	3.8	3.14	0.8	1.2	1.2	1.08
	710	2.8	3.0	3.2	3.01	0.6	0.8	1.1	0.83
	920	2.6	2.6	3.2	2.78	0.5	0.7	0.9	0.71
	Average	2.64	2.90	3.39	2.98	0.64	0.91	1.07	0.87
$\frac{3}{4}$	500	2.3	2.7	2.8	2.59	1.2	1.5	1.8	1.17
	710	1.9	2.4	2.7	2.33	0.8	1.3	1.5	1.22
	920	2.0	2.3	2.5	2.27	0.9	1.2	1.2	1.11
	Average	2.07	2.45	2.67	2.40	0.98	1.33	1.52	1.28
Average		2.32	2.76	3.18	2.75	0.77	1.32	1.65	1.24

Necessary difference (5% level of statistical significance) between averages of 3 = ± 0.30 .

Necessary difference (5% level of statistical significance) between averages of 9 = ± 0.17 .

Necessary difference (5% level of statistical significance) between averages of 27 = ± 0.11 .

TABLE III

INDEX OF SPATIAL TEMPERATURE VARIATION (STANDARD DEVIATION IN DEG. F.) UNDER SPECIFIED CONDITIONS OF DUNNAGE AND HEAT LOAD.

(AVERAGES FOR AIR FLOWS OF 500, 710, AND 920 C.F.M. VOIDS BLOCKED)

Stack heat load, B.t.u./hr.	Dunnage spacing, in.					
	0	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{3}{8}$	$\frac{1}{2}$	$\frac{3}{4}$
0	0.75	0.68	0.74	0.64	0.67	0.98
432	1.90	1.71	1.47	0.91	0.83	1.33
864	3.15	2.36	1.97	1.07	1.02	1.52

Necessary difference for 5% level of statistical significance = ± 0.28 .

spacings ranging from 0 to $\frac{3}{4}$ in., with the voids blocked. Under these conditions, optimum dunnage was clearly in the neighbourhood of $\frac{3}{8}$ to $\frac{1}{2}$ in., whilst the effect of reducing this spacing became progressively more pronounced with increasing heat load. Table IV and Fig. 2, D, constructed entirely from the second series of trials, show that increasing the air flow

TABLE IV
INDEX OF SPATIAL TEMPERATURE VARIATION (STANDARD DEVIATION
IN DEG. F.) UNDER SPECIFIED CONDITIONS
OF AIR FLOW AND HEAT LOAD.
(AVERAGES FOR 0, 1/4, AND 1/2 IN. DUNNAGE. VOIDS BLOCKED)

Air flow, c.f.m.	Stack heat load, B.t.u./hr.		
	0	432	864
290	1.25	2.12	2.94
500	0.92	1.58	2.41
710	0.65	1.32	1.90
920	0.60	1.31	1.84
1130	0.46	1.19	1.70

Necessary difference for 5% level of statistical significance = ± 0.23.

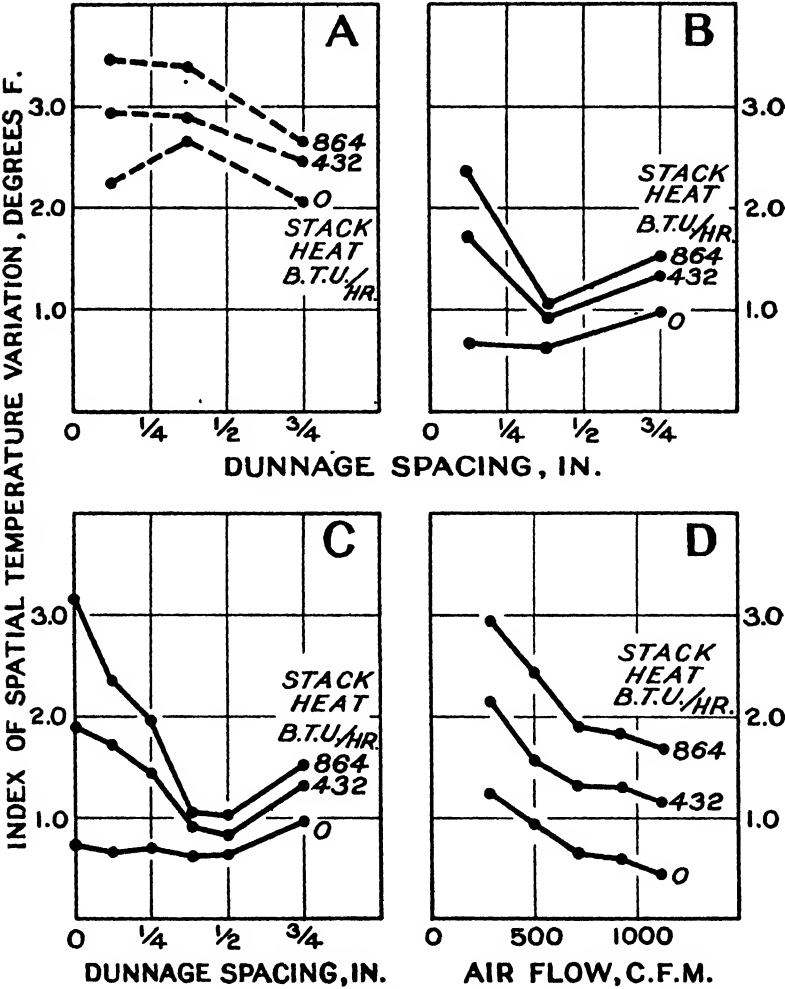


FIG. 2. Index of spatial temperature variation (standard deviation in deg. F.) in relation to dunnage, heat load, and air flow. A: voids open. B-D: voids blocked.

from 290 to 1130 c.f.m., i.e., from about 11 to about 40 changes per hour in the empty room, led to a progressive, although not directly proportional reduction in temperature variations. This was true of all the three heat loads and dunnage spacings included in this series.

Temperature Gradients

Systematic elements in the foregoing temperature differences within the stack were investigated by equating the mean temperature t characteristic of each of the 23 measured positions to a general quadratic function of the rectangular co-ordinates x, y, z of the point in question. This equation was of the form:

$$t = a + bx + cy + dz + exy + fxz + gyz + hx^2 + iy^2 + jz^2 + \epsilon \quad (1)$$

Values of the 10 coefficients $a-j$ were determined by the method of Least Squares so as to minimize in turn the sum of the squares of the residuals $S(\epsilon^2)$ for each of the sets of operating conditions included in the experiments. The difference between $S(\epsilon^2)$ and $S(t - \bar{t})^2$, where \bar{t} denotes the average of the 23 recorded mean temperatures, then provided a measure of the proportion of the total intra-stack temperature variance representable by continuous quadratic gradients. This is set forth in the form of percentages in Tables V to VII.

TABLE V
PERCENTAGE OF INTRA-STACK TEMPERATURE VARIANCE
REPRESENTABLE BY QUADRATIC GRADIENTS.
(AVERAGES FOR $\frac{1}{4}$, $\frac{1}{2}$, AND $\frac{3}{4}$ IN.
DUNNAGE AND AIR FLOWS OF 500,
710, AND 920 C.F.M.)

Stack heat load, B.t.u./hr.	Voids	
	Open	Blocked
0	88	90
432	88	92
864	86	91
Average	87	91

Augmented air flow (Table VI) or heat load in conjunction with $\frac{1}{4}$ in. or less dunnage (Table VII) resulted in some increase in the complexity of the heat distribution, which was accordingly less adequately represented by a quadric surface. In general nevertheless the quadratic function specified in Equation (1) accounted for some 90% of the total intra-stack variance, indicating that by far the greater part of the recorded temperature differences were associated with relatively simple continuous gradients, however different the form of these might be under the various conditions of heat load, dunnage, and air flow. It may be remarked that the terms in xy, xz , and yz in Equation (1) are to be interpreted physically as representing interactions of the linear temperature gradients in the x, y , and z directions. Such interactions would

TABLE VI

PERCENTAGE OF INTRA-STACK TEMPERATURE VARIANCE
REPRESENTABLE BY QUADRATIC GRADIENTS.

(AVERAGES FOR 0, $\frac{1}{2}$, AND $\frac{3}{4}$ IN. DUNNAGE. VOIDS BLOCKED)

Air flow, c.f.m.	Stack heat load, B.t.u./hr.		
	0	432	864
290	94	90	93
500	93	90	89
710	91	90	88
920	91	89	90
1130	81	89	87

TABLE VII

PERCENTAGE OF INTRA-STACK TEMPERATURE VARIANCE
REPRESENTABLE BY QUADRATIC GRADIENTS.

(AVERAGES FOR AIR FLOWS OF 500, 710, AND 920 C.F.M. VOIDS BLOCKED)

Stack heat load, B.t.u./hr.	Dunnage spacing, in.					
	0	$\frac{1}{8}$	$\frac{1}{4}$	$\frac{3}{8}$	$\frac{1}{2}$	$\frac{3}{4}$
0	92	92	96	87	86	90
432	89	88	91	93	91	94
864	89	86	85	94	93	94

operate to produce for example a modification of the longitudinal gradient as between the bottom and top or one side and the other of the stack. The occurrence of several such interactions was revealed by the analyses of variance. Their numerical magnitude was however in all instances definitely secondary to that of the average linear and quadratic trends.

Characteristics of Temperature Gradients

Tables VIII to X summarize the mean linear gradients deduced from the temperature differences recorded within the stack under various conditions. The mean longitudinal gradients listed range from $+0.64^{\circ}$ F. per ft. for a stack heat load of 864 B.t.u. per hr. with the voids open (Table VIII) to $+0.07^{\circ}$ F. per ft. for zero stack heat and 1130 c.f.m. air flow with the voids blocked (Table IX). In general the longitudinal gradient was reduced by more than 50% by blocking the voids or by increasing the air flow from 290 to 1130 c.f.m. It was rather less sensitive to variations in dunnage (Table X) but was at a minimum in the region of $\frac{3}{8}$ to $\frac{1}{2}$ in. The mean linear transverse and vertical gradients were for the most part small in magnitude and fluctuated irregularly in sign, indicating that the temperature differences recorded in these directions were distributed fairly symmetrically about the centre of the

TABLE VIII

MEAN LINEAR TEMPERATURE GRADIENTS (DEG. F. PER FT.). AVERAGES FOR $\frac{1}{8}$, $\frac{1}{4}$, AND $\frac{1}{2}$ IN. DUNNAGE AND AIR FLOWS OF 500, 710, AND 920 C.F.M.

Stack heat load, B.t.u./hr.	Longitudinal		Transverse		Vertical	
	Voids		Voids		Voids	
	Open	Blocked	Open	Blocked	Open	Blocked
0	+0.47	+0.13	+0.16	-0.03	-0.38	+0.01
432	+0.58	+0.24	+0.19	-0.06	-0.37	+0.04
864	+0.64	+0.34	+0.21	-0.10	+0.20	+0.06
Average	+0.56	+0.24	+0.19	-0.06	-0.13	+0.04

Necessary difference for 5% level of statistical significance: Longitudinal, ± 0.04 ; transverse, ± 0.02 ; vertical, ± 0.04 .

TABLE IX

MEAN LINEAR TEMPERATURE GRADIENTS (DEG. F. PER FT.). AVERAGES FOR 0, $\frac{1}{4}$ AND, $\frac{1}{2}$ IN. DUNNAGE. VOIDS BLOCKED

Air flow, c.f.m.	Longitudinal			Transverse			Vertical		
	Stack heat load, B.t.u./hr.			Stack heat load, B.t.u./hr.			Stack heat load, B.t.u./hr.		
	0	432	864	0	432	864	0	432	864
290	+0.27	+0.41	+0.54	-0.02	+0.02	+0.03	-0.11	-0.09	+0.27
500	+0.16	+0.29	+0.41	-0.02	-0.06	-0.05	-0.12	-0.03	+0.05
710	+0.18	+0.27	+0.34	+0.01	-0.04	-0.07	-0.07	+0.01	+0.01
920	+0.11	+0.22	+0.29	+0.00	+0.01	+0.02	+0.01	+0.02	+0.04
1130	+0.07	+0.21	+0.24	+0.00	+0.02	+0.07	+0.01	-0.01	-0.00

Necessary difference for 5% level of statistical significance; longitudinal, ± 0.07 ; transverse, ± 0.05 ; vertical, ± 0.14 .

TABLE X

MEAN LINEAR TEMPERATURE GRADIENTS (DEG. F. PER FT.). AVERAGES FOR AIR FLOWS OF 500, 710, AND 920 C.F.M. VOIDS BLOCKED

Dunnage, in.	Longitudinal			Transverse			Vertical		
	Stack heat load, B.t.u./hr.			Stack heat load, B.t.u./hr.			Stack heat load, B.t.u./hr.		
	0	432	864	0	432	864	0	432	864
0	+0.15	+0.29	+0.42	-0.10	-0.12	-0.14	-0.08	-0.08	-0.02
	+0.09	+0.25	+0.44	-0.10	-0.23	-0.36	+0.09	+0.10	+0.11
	+0.20	+0.32	+0.40	+0.05	-0.04	-0.04	-0.07	-0.05	-0.08
	+0.12	+0.21	+0.26	-0.01	-0.00	-0.01	+0.16	+0.06	+0.05
	+0.10	+0.17	+0.23	+0.05	+0.07	+0.08	+0.06	+0.08	+0.17
	+0.19	+0.26	+0.33	+0.03	+0.06	+0.08	-0.13	-0.08	-0.04

Necessary difference for 5% level of statistical significance: Longitudinal, ± 0.06 ; transverse, ± 0.06 ; vertical, ± 0.10 .

stack. They, however, present no information respecting the pronounced curvature actually characteristic of these gradients. This was examined analytically as described below.

If t is given some constant value such as $t_0 + 1$, where t_0 is the mean temperature of the cold air entering the room from the delivery duct, and if ϵ is neglected, then Equation (1) specifies the quadric surface approximating most closely to the actual surface generated by the aggregate of points within the stack at the temperature $t_0 + 1$. This may be termed the isothermal surface of $+1^\circ$. Similarly equating t to $t_0 + 2$, $t_0 + 3$, ---- $t_0 + n$ leads to the isothermal surfaces of $+2^\circ$, $+3^\circ$, ---- $+n^\circ$. Figs. 3 to 7 illustrate some of the characteristics of these surfaces under different experimental conditions by longitudinal, transverse, and vertical plane sections through the central axes of the stack.

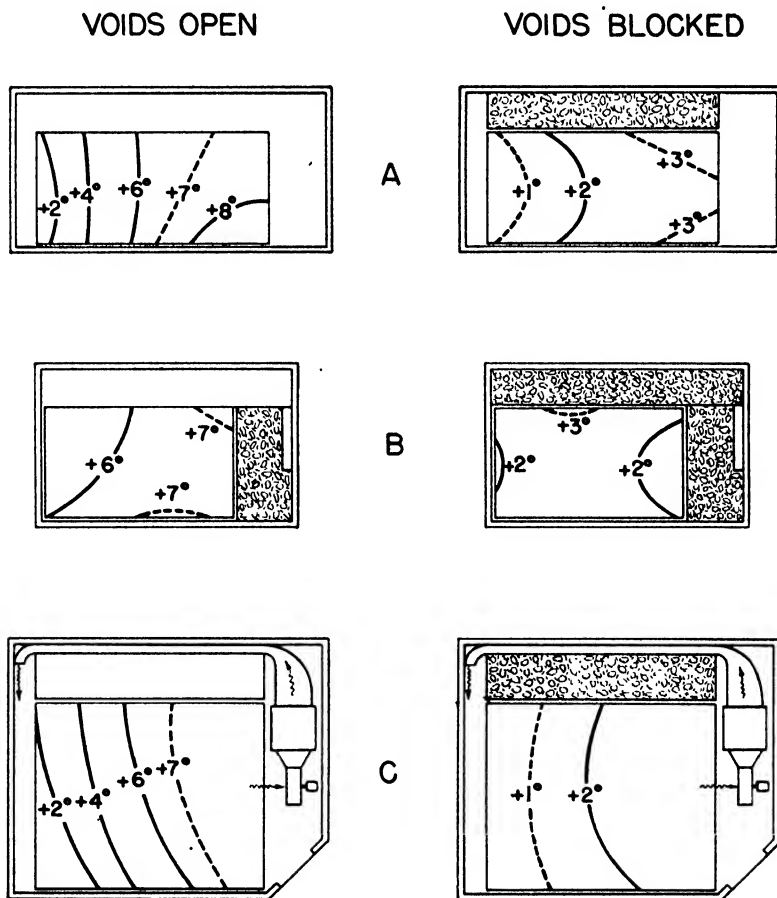


FIG. 3. Plane sections of isothermal surfaces. A: central longitudinal vertical section. B: central transverse vertical section. C: central longitudinal horizontal section. Averages for air flows of 500, 710, 920 c.f.m., heat loads of 0, 432, and 864 B.t.u. per hr., and $\frac{1}{8}$, $\frac{1}{4}$, and $\frac{1}{2}$ in. dunnage.

A considerable number of the surfaces obtained were sectors of hyperboloids of one sheet. They thus exhibited anticlastic ("saddle-shaped") curvature, presumably owing to the fact that cold air circulated across the sides of the stack more freely than across the top or bottom. However, the general breaking down of temperature gradients by an air flow of 1130 c.f.m. with the voids blocked resulted in an "open" isothermal surface for $+2^{\circ}$ F. which was approximated by the portion of a hyperboloid of two sheets illustrated in Fig. 6. Various segments of ellipsoids also resulted.

Fig. 3 portrays the marked decrease in both magnitude and complexity of temperature gradients resulting from blocking the voids and thereby channeling circulating air more effectively through the stack. Also to be noted is the asymmetry of the temperature gradients obtained with the void open. It would appear that in this circumstance, the non-central location of the discharge duct tended towards an orientation of the air circulation across and over the surface of the stack. This characteristic was largely, although not completely, eliminated by blocking the voids, the previously noted differential impedance of air movement across the top, bottom, and sides of the stack still remaining operative. The foregoing effects are observable in more detail in Figs. 4 and 5. These also illustrate the building up of temperature

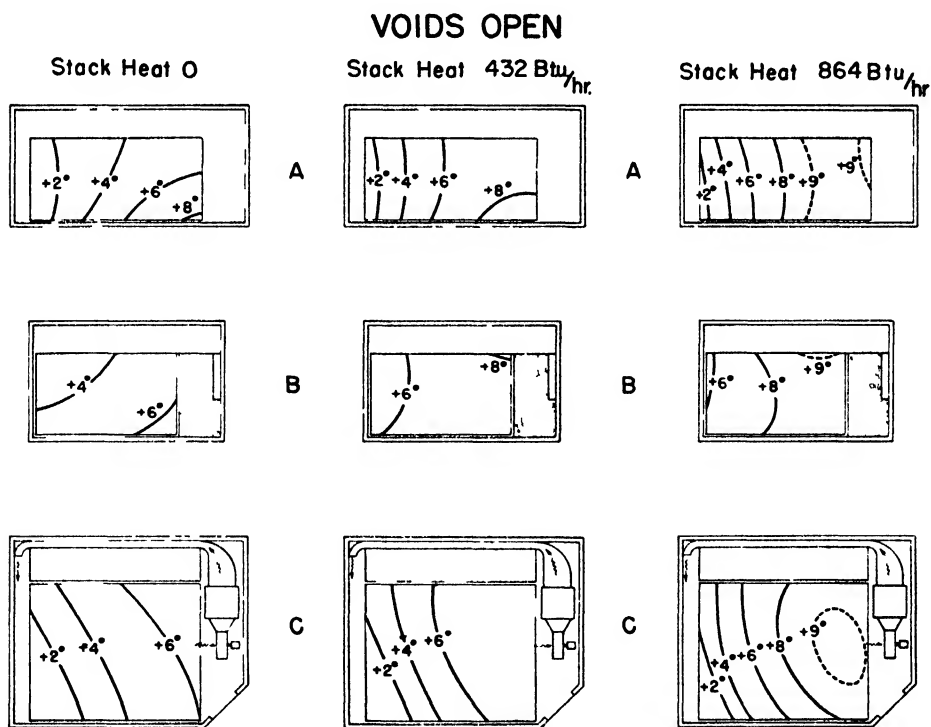


FIG. 4. Plane sections of isothermal surfaces, voids open. A: central longitudinal vertical section. B: central transverse vertical section. C: central longitudinal horizontal section. Averages for air flows of 500, 710, and 920 c.f.m. and $\frac{1}{8}$, $\frac{3}{8}$, and $\frac{1}{2}$ in. dunnage.

VOIDS BLOCKED

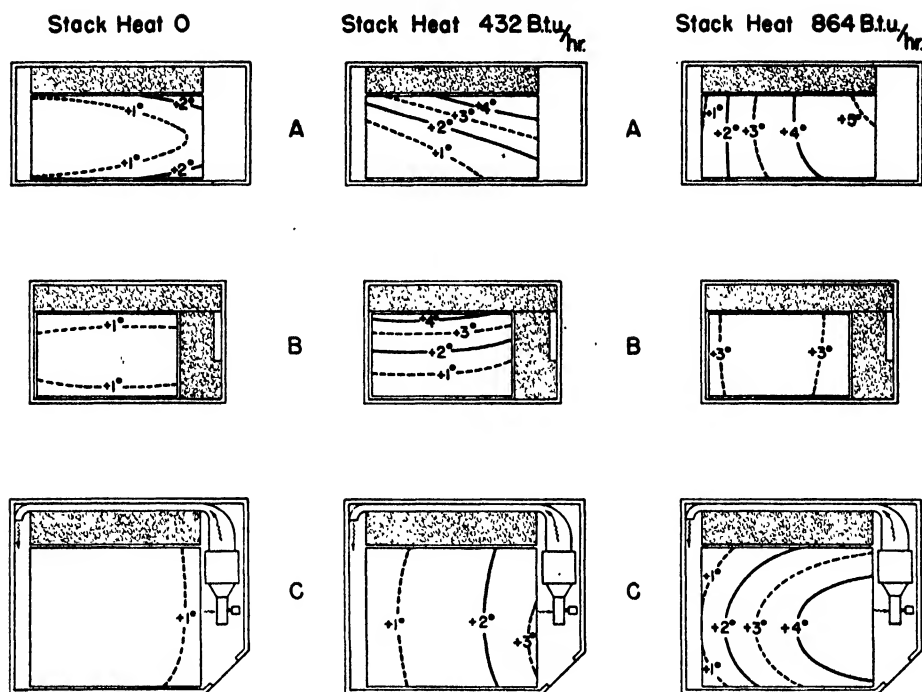


FIG. 5. Plane sections of isothermal surfaces, voids blocked. A: central longitudinal vertical section. B: central transverse vertical section. C: central longitudinal horizontal section. Averages for air flows of 500, 710, and 920 c.f.m. and $\frac{1}{8}$, $\frac{3}{8}$, and $\frac{1}{2}$ in. dunnage.

gradients by stack heat loads, whilst Fig. 6 exemplifies their reduction, particularly in the central zone of the stack, by augmented air flow. Fig. 7 shows the average gradients recorded with the voids blocked for zero, $\frac{3}{8}$ and $\frac{1}{2}$ in. dunnage, the second of these being in the vicinity of the optimum for the operating conditions of these tests.

This optimum was presumably occasioned by the balancing of two opposed factors as follows. Maintenance of an extra inch or $1\frac{1}{2}$ in. of free space in addition to dunnage at the top, bottom, and sides of the stack must have resulted in a correspondingly greater part of the total circulated air passing over these surfaces rather than through the apertures in the stack itself. This in turn would lead to relatively more accumulation of heat in the centre which, in the event of the stack apertures being small, would more than offset the effect on the external surfaces of radiation from the walls, ceiling, and floor. The left-hand side of Fig. 7 illustrates this effect in the extreme case of zero dunnage, i.e., no internal aperture at all. As the size of the internal apertures was increased, more air traversed the stack internally, and $\frac{3}{8}$ in. dunnage sufficed to reduce temperatures at the centre below those at the periphery, as shown in the middle portion of Fig. 7. On the other hand,

increasing the width of the apertures by additional dunnage must have correspondingly reduced the linear velocity associated with the circulation of a specified number of cubic feet of air per minute, until eventually a point was reached at which this became a limiting factor, and a further accumulation of

VOIDS BLOCKED

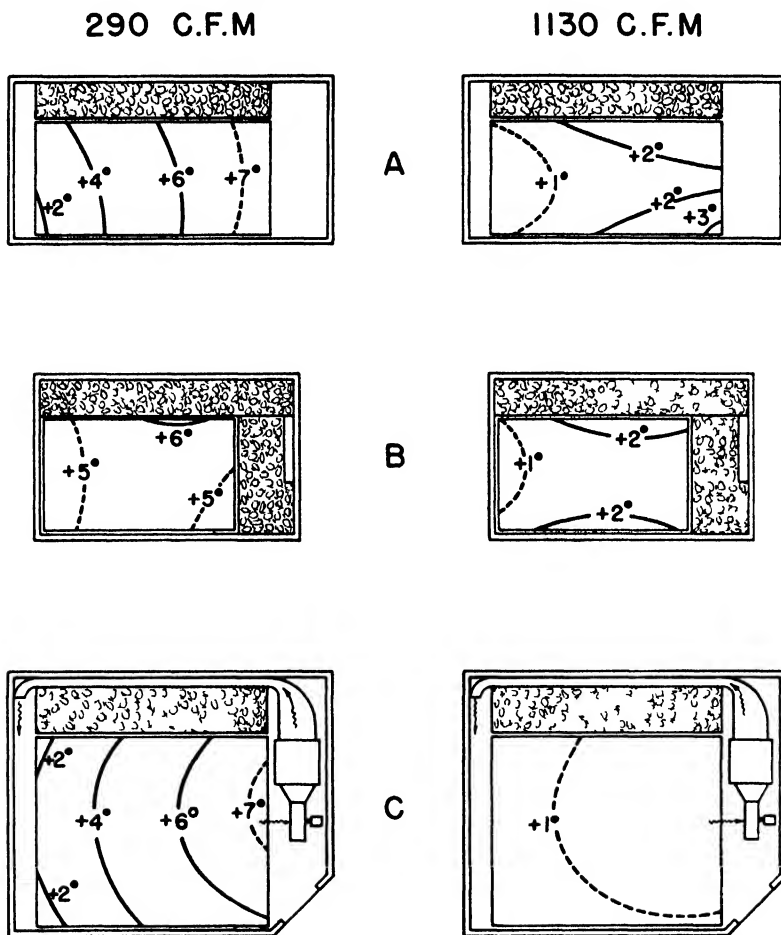


FIG 6 Plane sections of isothermal surfaces, voids blocked A central longitudinal vertical section B central transverse vertical section C central longitudinal horizontal section Averages for heat loads of 0, 432, and 864 B t u per hr

heat, particularly peripherally but to some extent internally as well, became manifest This would appear to be the situation exhibited in the right hand portion of Fig 7

It will be appreciated that the quadric surfaces delineated in Figs 3 to 7 are only second-degree approximations to the actual isothermal surfaces generated within the stack From Tables V to VII, however, it is clear that

they must in fact portray fairly closely most of the recorded temperature gradients. In general these gradients were characterized by more pronounced curvature in the vertical than in either the horizontal or transverse planes,

VOIDS BLOCKED

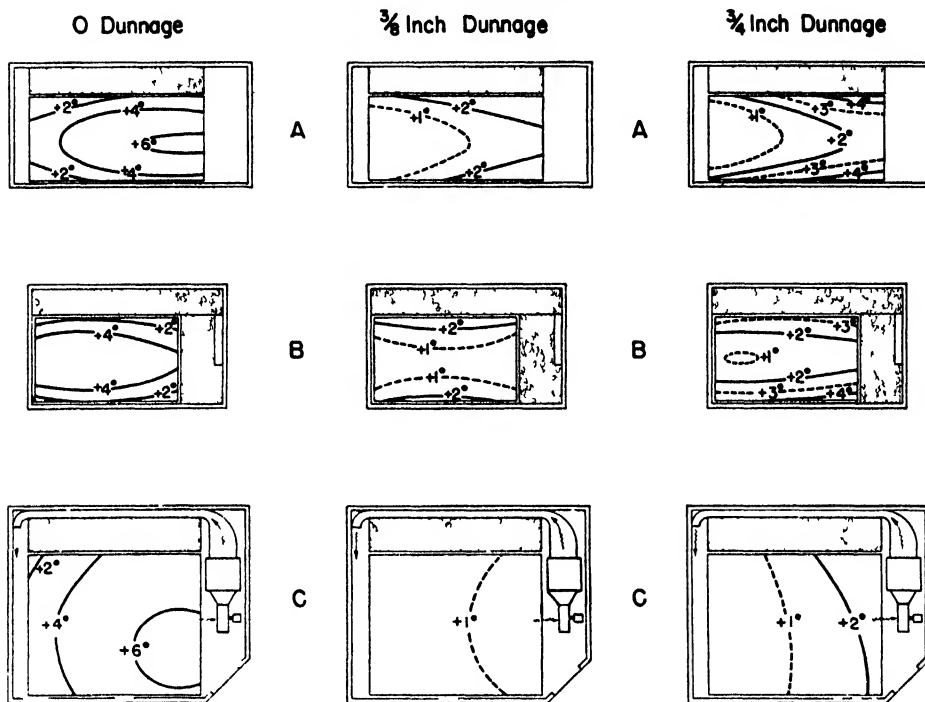


FIG 7. Plane sections of isothermal surfaces, voids blocked A: central longitudinal vertical section B: central transverse vertical section C: central longitudinal horizontal section Averages for heat loads of 0, 432, and 864 B t.u. per hr. and air flows of 500, 710, and 920 c f.m

presumably as a further consequence of the unequal restriction of air movement over the top, bottom, and side surfaces. Dunnage eliminating vertical and transverse gradients and reducing all isothermal surfaces to planes at right angles to the direction of air flow would be desirable as indicative of uniform effectiveness of heat removal throughout the stack. An approximation to this might perhaps be achieved by using different widths of dunnage at the centre, periphery, and exterior, although the requirements would almost certainly be modified by any alteration in the size or shape of the stack or in the location of inlet and exhaust ports.

Conclusions

It is concluded that these experiments were in agreement with previous work in this laboratory in demonstrating (a) the occurrence of appreciable permanent temperature gradients in material, whether exothermic or not,

stacked in an air-cooled storage room, and (b) the possibility of markedly reducing these gradients by effective channelling of air through the stack. With end-to-end circulation, blocking of voids in the room was the most important single factor in minimizing intra-stack temperature differentials under the conditions of these tests. Further improvement was effected by the provision of optimum dunnage and by augmenting the air flow. It is to be inferred that with blocked voids, dunnage should be extended to all external surfaces of the stack. The desirability of uniform transverse and vertical distribution of the circulating air was also evident. Further trials on a larger scale are required to investigate the extent to which the present results were a function of the size and shape of the stack, and also to determine the most suitable ratio of external to internal dunnage.

Acknowledgments

A. E. Chadderton rendered technical assistance in the conduct of the experiments, whilst W. R. Coutts, M. J. Mahoney, F. H. Smith, and Mary Wall participated in the statistical treatment of the results.

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A CLINOMETER FOR MEASURING SMALL ANGLES¹

By R. H. FIELD²

Abstract

The paper describes a clinometer for the accurate measurement of angles of elevation or depression up to 5° from the horizontal. The geometrical design permits a theoretical precision of $\pm 2''$ to be attained in this range on a uniformly divided micrometer head without the application of corrections or the use of cams or associated devices. While the instrument can be read to $1''$ and is sensitive to about twice this amount, no special tools or jigs were needed for its construction, beyond the equipment ordinarily available in a small machine shop.

Purpose

During the adjustment and calibration of certain types of fire-control equipment, it is necessary to measure small angles of elevation or depression (usually less than 5°) to a precision of two or three seconds of arc. In the Canadian services it has been customary to rely principally on the old Watkin type clinometer for all angle measurements in a vertical plane; but this instrument is subject to the acquisition of serious periodic errors, and it was probably never expected to work to the precision under consideration. The new clinometer, described below, filled the need, and has been found quite suitable, for example, in calibrating or forming correction elements provided in fire-control apparatus to compensate for the curvature of the earth. For this work, hitherto, somewhat elaborate collimator or other equipment had been necessary.

Principle

The micrometer screw is the usual element employed in a self-contained clinometer for the measurement of small angles. Kinematically, the design of this class of clinometer can be considered by reference to a simple triangle, ABC , Fig. 1. The side a represents the contact plane which rests on the

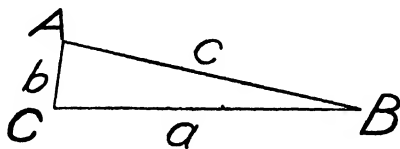


FIG. 1.

surface of which the inclination to the horizontal is to be measured. A level vial is attached to the side c which is brought to the horizontal by means of a micrometer screw represented by the side b . B is the measured angle.

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The designer has the choice of various possibilities. If C be made and fixed as a right angle, c remain of constant length and the nut be fixed to c , a uniformly graduated micrometer head on b will actually indicate, to some scale, the quantity $\sin B$. Consequently, if the scale be adjusted to read angles correctly at 0° and 5° , the theoretical error in the indications (nominally subdivided equally in angle) will be at any measured angle θ :

$$\text{Error} = 18,000 \operatorname{cosec} 5^\circ (\sin \theta - 0.2 \theta^\circ \sin 5^\circ) \text{ seconds of arc.}$$

This error is shown graphically in Curve A , Fig. 2. The Watkin clinometer has a vertical, fixed, micrometer screw, but the side c , Fig. 1, is variable in length, and the graduations on the micrometer drum are not uniform, so that the simple theory does not apply.

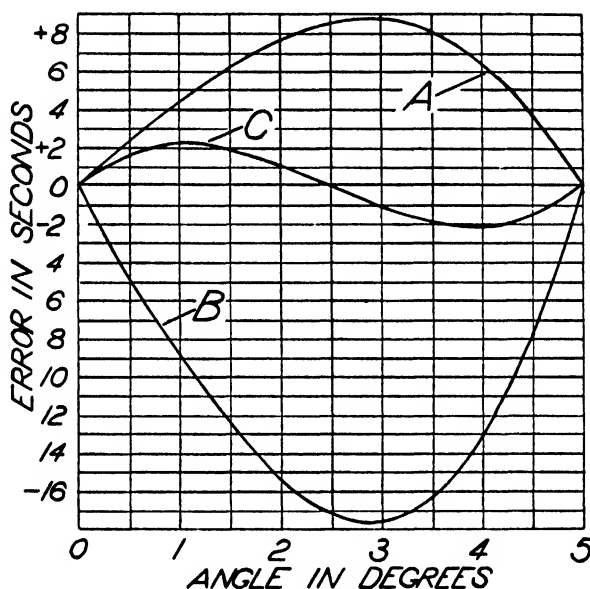


FIG. 2.

As an alternative, A may be fixed at 90° and b hinged at C while c still remains constant in length. In this case the indications will be proportional to $\tan B$. A clinometer of this form, adjusted at 0° and 5° as before, would indicate angles with an error:

$$\text{Error} = 18,000 \cotan 5^\circ (\tan \theta - 0.2 \theta^\circ \tan 5^\circ) \text{ seconds.}$$

This error is shown graphically in Curve B , Fig. 2.

Both curves, A and B , show errors undesirably large for the purpose in view, and a modification of the second scheme was considered in which the angle A was made 87.5° , b was hinged at C , and the end of c moved along a at B . The simple analysis showed that this design, for a range of 5° , should make possible a maximum error of $\pm 2''$ (i.e., corresponding to two-thirds

of an inch in one mile) in the readings of a uniformly graduated micrometer drum, compared with respective maxima of $+9''$ and $-17''$ for the two cases previously considered. Actually, the theoretical error in the third case, with the same provisions as before would be:

$$\text{Error} = 18,000 \operatorname{cosec} 5^\circ \cos 2^\circ 5' \{ \sin \theta \sec (2^\circ 5' - \theta) - 0.2 \theta^\circ \sin 5^\circ \sec 2^\circ 5' \} \text{ seconds.}$$

Description

In order to gain advantage of the attainable precision indicated by the small theoretical error, care was taken during the mechanical design to reduce the possibility of secondary errors arising from the functioning of the parts. A complete clinometer, as finally designed by the author and built in the Instrument and Model Shop of the National Research Laboratories, Ottawa, is shown in Fig. 3. Fig. 4 is a "close up" of the micrometer screw end of the instrument, while Figs. 5 and 6 are drawings to illustrate details.

Base

The base is a bronze casting, shaped at the bottom to give a bearing surface (scraped flat) of 2 by $6\frac{1}{4}$ in., to conform to Army requirements. The centre portion is cored to save weight and the ends are machined for the fittings described below. Guards are screwed to the micrometer end and the two sides of the base to protect the measuring parts against damage.

Inclinable Platform

This is also a bronze casting, bearing two posts for carrying the level vial housing, one end of which is tapped for two opposing screws, bearing against the post for windage adjustment. The vial has a value of 10 sec. per division. At the hinge end of the platform a longitudinal groove is machined to locate the hinge piece, and the other end is bored and split to receive the nut.

Micrometer

Referring to Fig. 5, *A* is the steel micrometer screw, which is 0.56 in. diam. and has 40 t.p.i. It works in the split nut, *B*, 1 1 in. long. Screw *A* is bored and reamed up the centre and at the end of the reamed hole a hard steel piece, *C*, is inserted, having its lower face flat and polished. This face rests on the steel bearing ball, *D*, peened into the centre of the top of the pivot, *E*, which is a good fit in the axial hole in the screw. To free *E* from constraint as to inclination, its lower end is made spherical in shape, and rests in the cup, *F*. A pin, *G* engaging in a slot in the spring-loaded cover, *H*, prevents axial rotation of *E*. The micrometer drum is held in place by friction when the finger knob, *I*, is tightened by the central screw tapped into the top end of *A*. This permits the zero of the clinometer to be adjusted at any time by slackening the central screw and turning the drum; which is to be preferred over adjustment involving a change in the level vial with respect to the inclinable platform, owing to the inevitable bubble creep that takes place after straining the

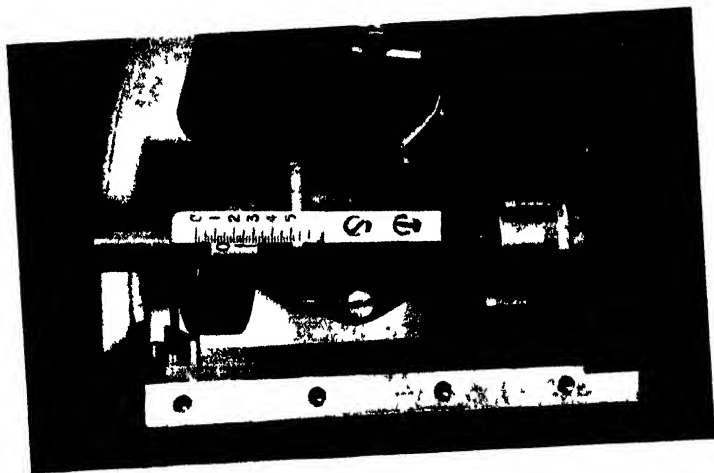


FIG 4

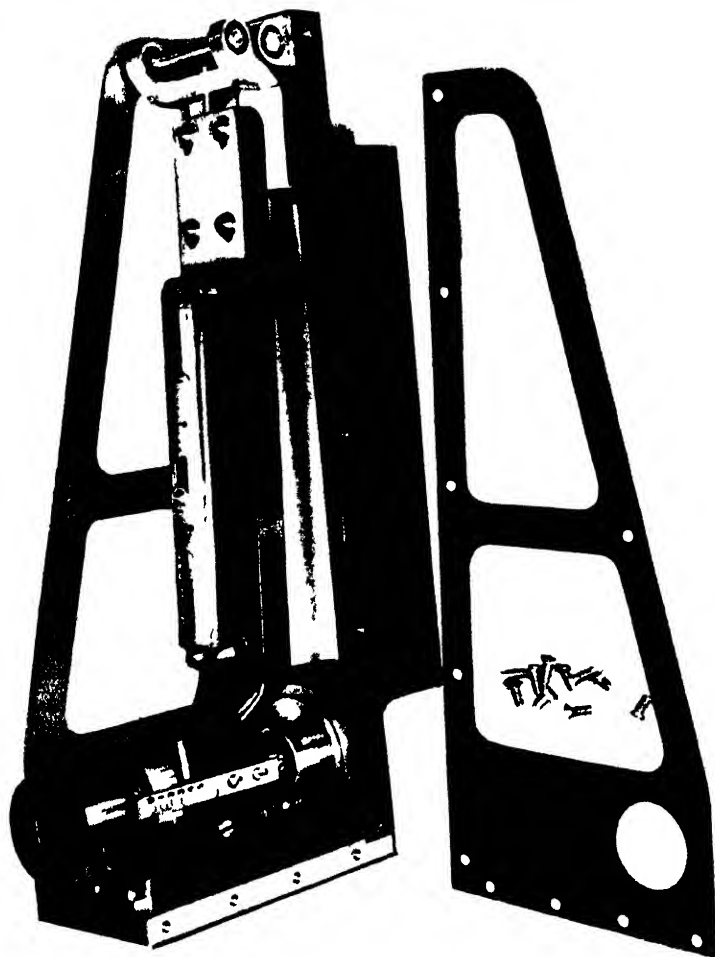


FIG 3

FIG 3 Sensitive clinometer, one side of the guard is removed to reveal details
 FIG 4. Micrometer screw end of sensitive clinometer In this instance one division on the drum corresponds to 0 2', but later instruments have been made to read directly to 0 1'

level-mounting screws. To prevent the micrometer screw from lifting off the pivot, two leaf springs, *J*, bear on a fibre washer on the top of the micrometer drum. The length of the effective lever arm of the clinometer is made and adjusted so that one turn of the micrometer screw corresponds to an angle of 10 min. The drum is divided into 10 major 1' divisions, and each minor subdivision represents 0'.2. In later instruments the subdivisions have been made 0'.1 in value, and by estimation readings are easily made to 0'.02 (or 1").

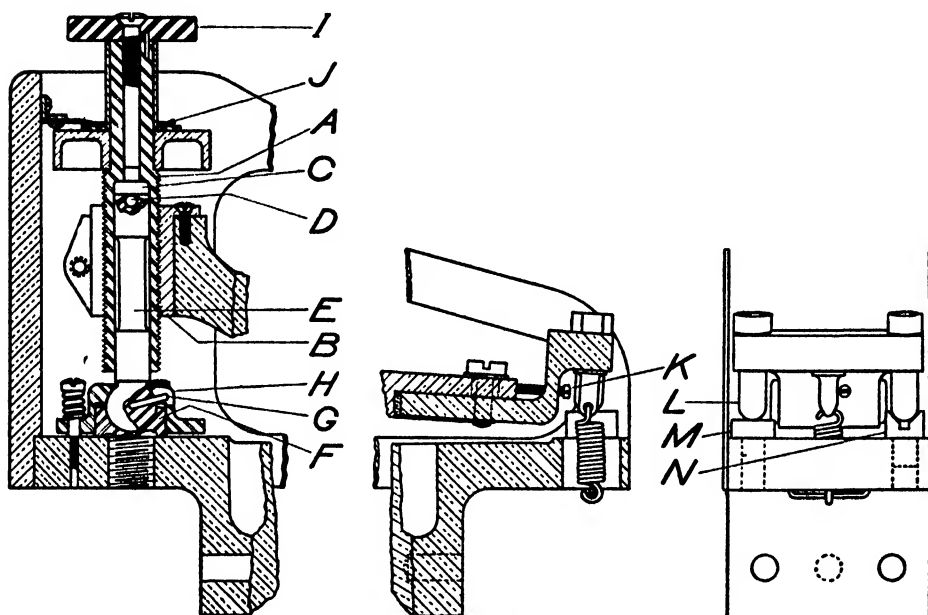


FIG. 5.

Hinge

To facilitate the adjustment of the effective lever arm, the hinge piece is attached to the inclinable platform by screws passing through slotted holes in the platform. A screw, *K*, tapped in the hinge piece, with the end contacting the platform, was found very convenient in adjusting the clinometer in the laboratory.

In the first model the hinge piece was fitted with two steel pins, *L*, having hardened hemispherical ends; one pin resting on the hard flat insert, *M*, and the other on the grooved insert, *N*. Closure was maintained by a coil spring. In a later model, an alternative device, Fig. 6, is used. The hinge piece and the base are both fitted with housings for 0.375 in. O.D. ball bearings. A link, *O*, has four round portions turned on it, each to fit the bore of the bearings and so as to yield two horizontal axes spaced 0.8 in. apart. This later form of hinge is cheaper, and as the bearings can be packed with, say, vaseline,

it is thought there is less possibility of troubles due to corrosion. Experience alone will show whether there is any advantage of one form of hinge over the other.

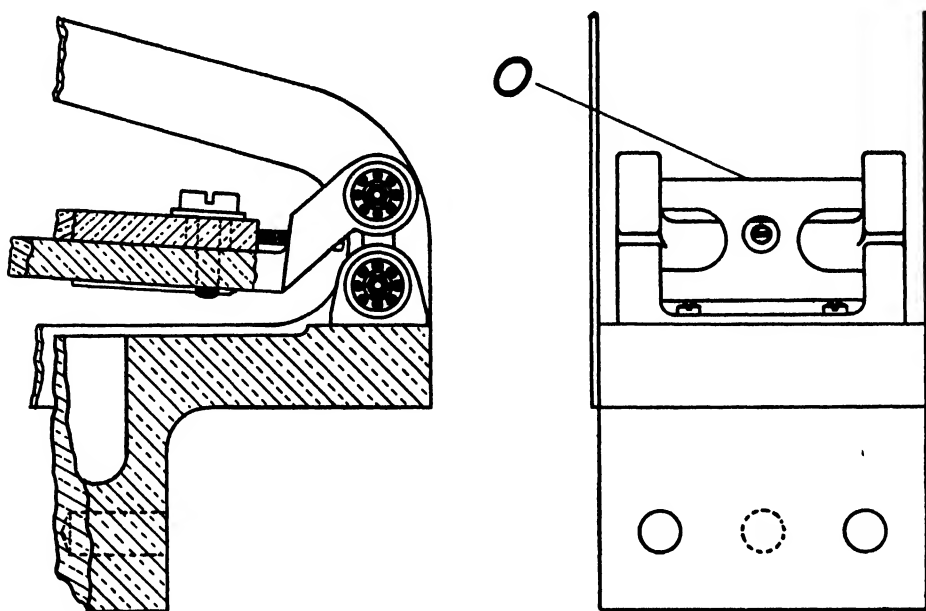


FIG. 6.

Construction and Calibration

The construction in the shop presented no difficulties. Care was taken to cut the screws on a good lathe and both screws and nuts were lapped to spare units before running them together.

For the adjustment and calibration use was made of an angle-head reading to seconds, and described in a separate paper (1). First the effective length of the inclinable platform was adjusted by trial to give the minimum corrections over the range 0 to 5°. Then a check was made at each 30' interval in this range, together with a test at each 0.2' throughout one complete turn of the micrometer screw at 0°, 2.5°, and 4°. From repeating the tests, it would appear that these clinometers can be adjusted to yield uncorrected readings accurate to 5'', while with the application of corrections and the exercise of care as to alignment and other precautions inherently necessary in any method of measuring angles to the precision of a few seconds, angles can be measured to a precision of 2 or 3''. The calibration corrections for one of these clinometers, determined when new, are given below. This particular instrument was damaged in Army service, and after repair and recalibration the corrections were found to agree to $\pm 2''$ with the original ones, determined some four years earlier.

SENSITIVE CLINOMETER No. 2

Calibration corrections

Error in seconds of arc at given readings										
Scale reading	Micrometer drum reading									
	0.0	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	0.9
0° 00'	0		+2		+2		+1		+2	
0 30	0									
1 00	+2									
1 30	+2									
2 00	+1									
2 30	0	+2		+3		+3		+1		
3 00	-2									
3 30	-2									
4 00	-2		-2		-3		-5		-4	
4 30	-1									
5 00	0									

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TWO ACCESSORIES FOR THE ELECTRON MICROSCOPE LABORATORY—(A) SPECIMEN STAGE (B) PLATE READER¹

By W. M. BARSS²

Abstract

A new electron diffraction specimen stage is described which may be used in place of the standard RCA diffraction stage to permit five independent transmission type diffraction patterns to be obtained without opening the vacuum system. The modification of a commercial microfilm reader for use in measuring electron diffraction patterns is also reported.

I. A Multiple Diffraction Specimen Stage for the Electron Microscope

In a series of experiments involving the identification of powdered materials by electron diffraction pictures of the transmission type it was found desirable to load several specimen holders at once and to provide means for interchanging them inside the vacuum system. Fig. 1 illustrates the design of a multiple specimen stage constructed to replace the diffraction specimen stage supplied with the RCA Type EMU-1 electron microscope, and Fig. 2 shows its external appearance.

Parts corresponding to RCA stock parts for the regular stage were used whenever possible: this applies particularly to the design of the external and internal vacuum seals. The slide *a* carries five of the standard transmission apertures and specimen holders and moves horizontally in a slot in the supporting piece *b*, which is held in the correct position in the stage body *c* by means of a set screw and the aligning key *d*. The slide is moved by the pin *e* in the end of the push rod *f* and is positioned by a spring-loaded detent *g* engaging notches in the edge of the slide. The handle *h* aids in inserting the stage into the microscope. The parts are of brass with the exception of the rubber vacuum gaskets and the steel push rod, key, ball bearing, and two compression coil springs. External surfaces are nickel plated.

The device has been found to operate well. It does not affect the evacuation time of the microscope appreciably, and the lubricated rubber packing provides a satisfactory vacuum seal for the well polished push rod. The holes and notches in the slide are located with sufficient accuracy that it is unnecessary to adjust the electron beam position when specimens are changed. Since a standard specimen such as gold leaf may be kept in one of the five specimen holders and its diffraction pattern obtained under conditions identical with those for the other specimens, it is convenient to register on each plate a standard pattern to be used for calibration.

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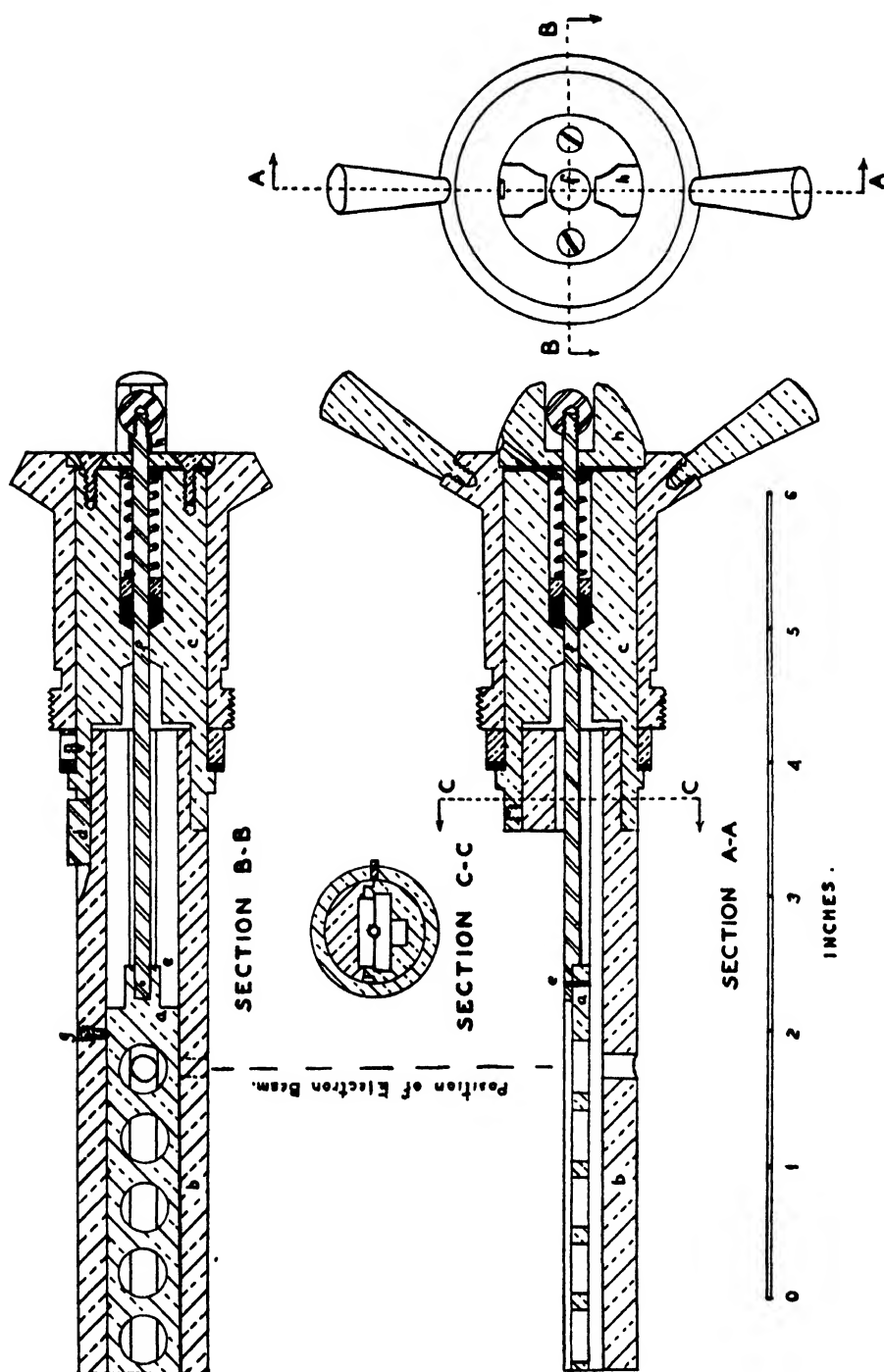


FIG. 1. Multiple diffraction specimen stage, sectional drawing.

II. Projector for Electron Diffraction Patterns

The electron diffraction patterns obtained with the RCA Type EMU-1 electron microscope are so small that it is difficult to measure ring diameters with as great accuracy as desirable without using a magnifier of some kind. The type of comparator commonly used for measuring spectroscopic plates has too high a magnification for this service, as the field of view is too small and the visibility of weak rings is greatly reduced. It has been found that slight modification of a commercial microfilm reader gives an instrument that projects a magnified image of the diffraction pattern upon a translucent screen, where measurements may be readily made with a ruler.

The microfilm reader used for this purpose was made by the Society for Visual Education, Chicago, a portable type, RM, which uses two mirrors to throw the projected image upon the ground glass screen forming the front of the case. The film reels and film advance mechanism were replaced by an adjustable frame into which the 2 by 10 in. plates used in the electron microscope may be inserted. The original lens (approx. 50 mm. focal length) was replaced by an 80 mm. lens (a 3 in. projector lens is equally suitable) giving a magnification of about $7\frac{1}{2}$ times. The aperture of the lens system is not sufficiently large to cover the whole area exposed in the microscope but does cover a circle of diameter approximately 4 cm., which is adequate for most work.

In order to minimize distortion in the projected image, the effective axis of the projection lens, as reflected by the mirrors, should fall normally upon the viewing screen. It was found that the axis could be made to lie in a vertical plane through the centre of the screen, but not readily in the normal plane having a horizontal intersection with the screen at its centre. Because of the resulting distortion in the vertical direction all patterns to be measured are made concentric about a mark at the centre of the screen, and measurements are made along the horizontal diameters of the rings.

When a diffraction adapter was introduced for the first RCA electron microscopes it was pointed out that the smallness of the angles involved permitted reduction of the diffraction equation to the simple form $Dd = 2k\lambda L$ (2), where D is the diameter of the diffraction ring corresponding to the lattice spacing d , L is the distance between the specimen and the photographic plate, λ is the de Broglie wave-length of the electrons, dependent upon the accelerating voltage, and k is an instrumental constant dependent upon the diffraction lens power. It is convenient to combine the factors on the right-hand side of the above equation into a single quantity K which will remain constant as long as specimens are placed in the same position, the high voltage does not vary, and the lens setting is unchanged. It has become the practice in this laboratory to expose a standard diffraction specimen, usually gold, with each group of other specimens in order to check the calibration and find the amount of variation to be expected in the value of K .

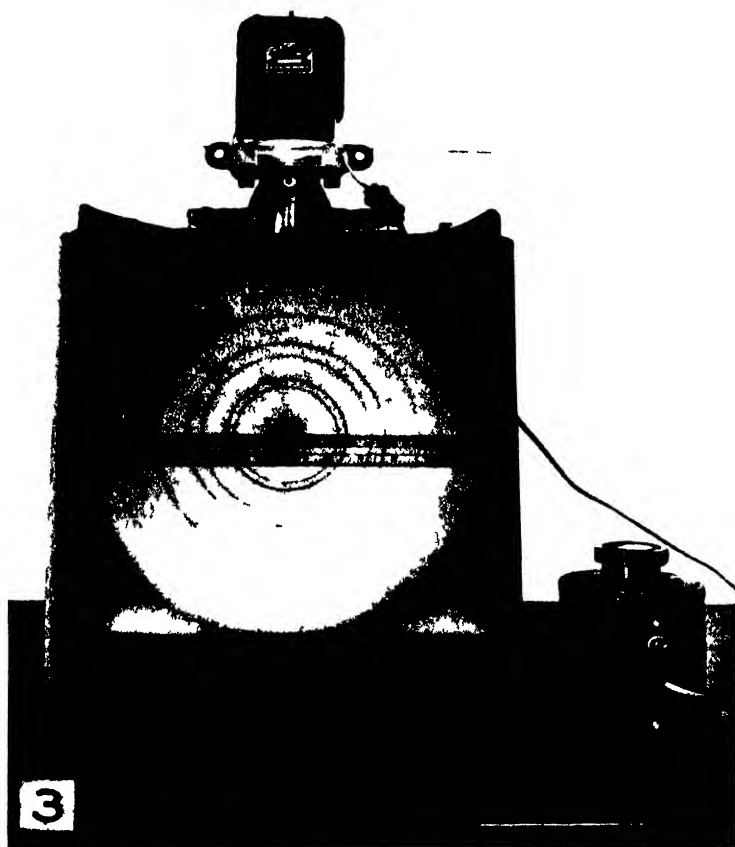
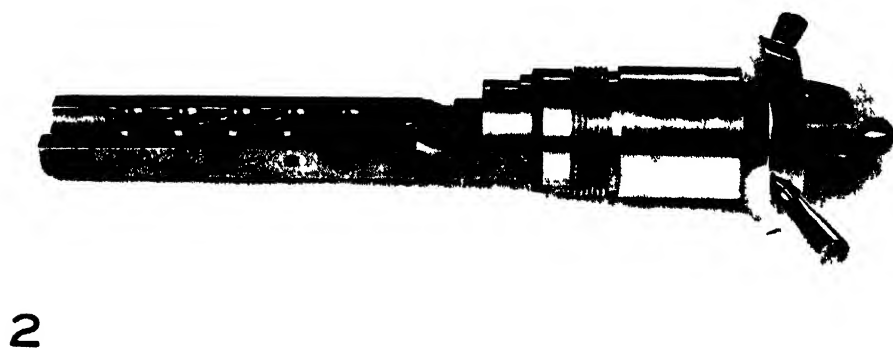


FIG. 2 *Multiple diffraction specimen stage*

FIG. 3 *Projector for electron diffraction patterns*

The value of the product $D \times d$ has been found to change slightly for larger values of D because of the inaccuracy of the approximation for larger diffraction angles, and possibly because of distortion introduced by the lens used in measuring the plates. A diffraction pattern of gold was therefore measured over as wide a range of ring diameter as possible. Results are given in Table I. The lattice spacings were calculated from the lattice constant $a = 4.0702 \text{ \AA}$ (1).

TABLE I
MEASUREMENT OF GOLD DIFFRACTION PATTERN (PROJECTED)

Reflection indices, hkl	Lattice spacing d in \AA (10^{-8} cm.)	Ring diameter, D , cm.	Product $D \times d$ in 10^{-8} cm. ²
111	2.350	7.75	18.21
200	2.035	8.95	18.21
220	1.438	12.65	18.19
311	1.226	14.85	18.21
222	1.174	15.50	18.20
400	1.017	17.90	18.20
331	0.934	19.50	18.21
420	0.910	20.00	18.20
422	0.831	21.9	18.20
333/511	0.784	23.2	18.19
440	0.719		
531	0.688	26.3	18.1
442/600	0.678		
620	0.643	28.2	18.13

Average value for first 10 rings, $K = 18.202 \pm 0.003$

The values of $D \times d$ are seen to be quite consistent up to projected ring diameters of about 25 cm., and the deviation is only about 0.5% for the largest rings. Experience has shown that lattice spacings of unknown materials may be calculated, using such a calibration, with an error of about 0.5% or better.

Acknowledgments

The author wishes to acknowledge the assistance of Mr. H. A. MacDonald in the design and construction of the multiple diffraction specimen stage.

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RECENT DESIGN OF A SMALL TELEPHONE MAGNETO¹

By D. S. SMITH²

Abstract

An example is given of the product obtained by applying modern methods in permanent magnet design to the problem of constructing a hand driven magneto of about flashlight battery size. The method of arranging the mechanical parts to conform with the electromagnetic system is described.

During the past few years, there have been tremendous improvements in permanent magnet materials. These have been closely followed by improved design methods. Thus it is now usually possible to predetermine closely the characteristics of a device using a permanent magnet in its magnetic circuit, and to design the device for minimum magnet size by direct, as opposed to cut-and-try methods (1).

An interesting problem in the design of such a device for use in a rather special application was presented to the author in the form of a request for a telephone magneto to meet very limited space requirements. The dimensions and output called for were as follows:

Outside diameter	1 7/16 in.
Length	2½ in. approximately
Output	10 v. minimum with a load of 500 ohms.

It will be noted that this is very little larger than an ordinary flashlight battery. The small diameter presented a problem and first rough designs on paper were most unattractive. However, it soon became apparent that the use of two annular magnets with the armature in between would result in a workable design, and, once the general physical arrangement was settled upon, a detailed design soon followed.

Fig. 1 shows details of the pilot model, which differed only in minor details from the initial design. The two magnets are Alnico II and are of manufacturer's stock size. The remainder of the magnetic circuit (the two pole pieces and the rotor) is of ordinary mild steel. The two bearings are of the Oilite type and are pressed into the magnets. To simplify winding of the armature two stub shafts are used instead of a through shaft. These stub shafts are spigotted to the armature and held with screws.

The rotor is driven manually through an epicyclic gear train with a drive ratio of 5.8/1. Some difficulty was encountered at first when using a single planetary gear. This was satisfactorily overcome by using three planetary gears, thus eliminating any tendency for the gears to bind.

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The two pole pieces are pulled tight over the magnets by means of the four brass screws, which also serve to hold the complete assembly together. The magneto was designed to fit into a non-magnetic case with a screw-on end-cover. The handle and planetary gear assembly are mounted directly on the end-cover, as shown in Fig. 1.

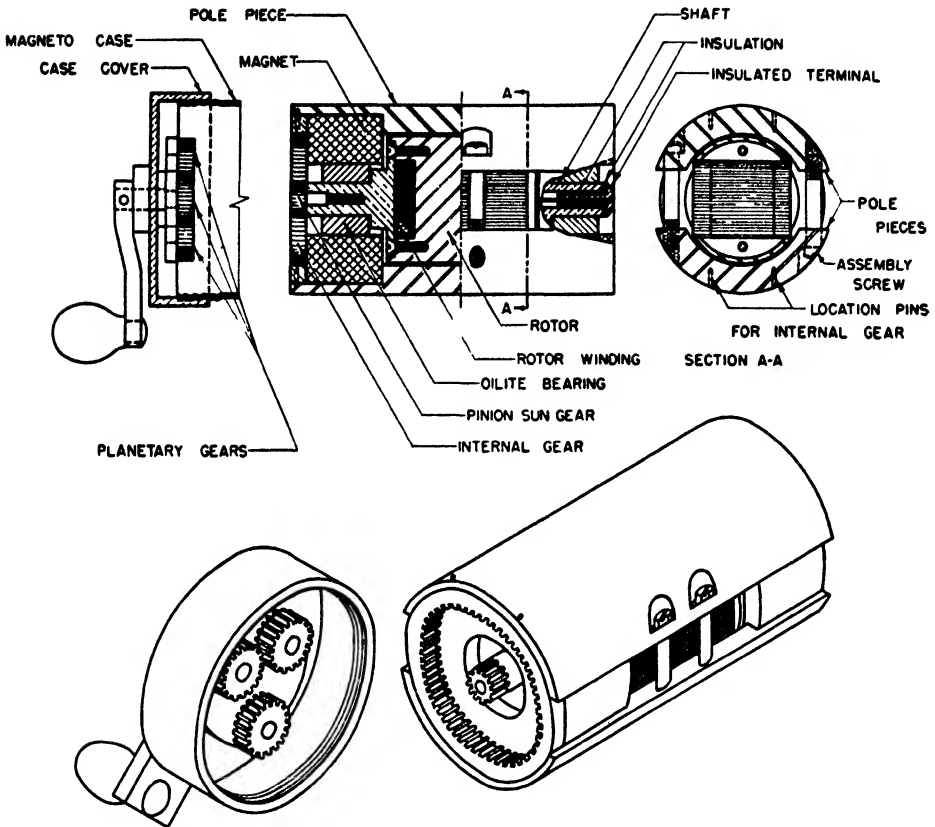


FIG. 1. Sectional and isometric views of the complete assembly.

The design of the magnetic circuit was determined to a very marked extent by the over-all dimensions and further by the dimensions of available magnets. It was desirable that the magnets as finally used have almost the same dimensions as a type available commercially, since any excess material must be removed by grinding. Apart from these considerations the peculiar configuration of the magnetic circuit made design to usual criteria difficult. In spite of this the output was predetermined with a reasonable degree of accuracy, after somewhat arbitrary allowances were made for leakage.

The permanent magnet alloy, Alnico II, was chosen because its high energy content and good remanence characteristic permitted a small volume of the material to deliver the required working flux. In addition, it was desirable that the material used have resistance to the demagnetization caused by stray

fields, vibration, shock, and temperature changes. The high coercive force characteristic of Alnico II makes it particularly suitable for such an application.

Data on probable load resistances indicated that these might range from 500 to 10,000 ohms, and it was decided to design for maximum power output in the region of 500 ohms. This resulted in the use of approximately 3500 turns of No. 38 wire.

Magnetizing was undertaken in a specially built magnetizer with poles fitting closely over the pole pieces of the magneto. The positions of the poles and the flux paths after removal of the magnetizer are shown in Fig. 2.

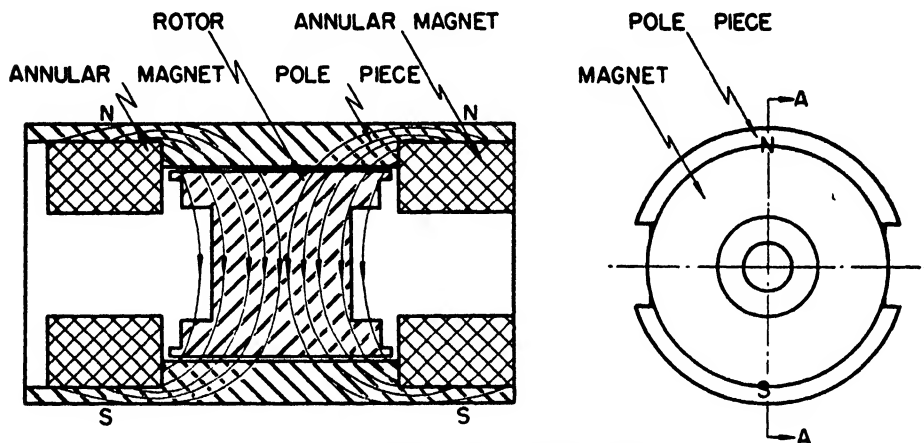


FIG. 2. Diagram of magnetic flux paths.

The design called for a flux density of 6000 lines per sq. cm. at the neutral section of the magnet. It was not considered necessary to stabilize the magnets after magnetizing, since a higher than normal initial voltage presented no disadvantage. In the initial state of magnetization and at a handle speed of 100 r.p.m. the output was 30 v. on open circuit. After allowing for the reduction in output that occurs as the magnets become stabilized, the capacity of the magneto is still easily capable of meeting the operating requirements.

An interesting sidelight was the subsequent discovery of a very similar piece of German equipment that was apparently used as a generator with a tachometric gun-sight. The general configuration of the magnetic circuit was identical, differences being largely in minor details. For instance, a non-magnetic band, crimped in place was used to hold the assembly together. This method was first considered for the design described here, but later discarded in favour of the assembly screw method shown above.

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PACKAGING

IV. METHODS OF APPLYING WATER-VAPOUR BARRIERS, AND THE WATER-VAPOUR RESISTANCE OF SOME PACKAGING MATERIALS¹

BY C. G. LAVERS² AND JESSE A. PEARCE³

Abstract

Reynolds' Metal A-10 and 450 M.S.Y.T. "Cellophane" were used as liners and overwraps and Darex P 16 as a wax-dip for cartons containing sawdust, and packed in a master container. Some packages were dropped a distance of three feet, 20 times at -40° F., others received the same treatment at room temperature, and some were subjected to a free fall of about 70 ft. Greatest protection was provided by the use of a liner inside the carton.

Water-vapour resistance and ability to withstand rough handling were investigated for a wide variety of packaging materials (all materials but one tested as carton liners). Laminated materials having metal foil as one layer provided the greatest protection. Wax-coatings effectively reduced water-vapour transmission, but provided little added protection when packages were subjected to shock. Laminating two stocks produced marked reduction in the water-vapour transmission typical of either base sheet when used alone. Combinations utilizing scrim or kraft produced barriers that were less likely to fracture when subjected to rough handling. When Cellophane was considered, M.S.Y.T. stock and the use of triplex bags provided the greatest protection.

Introduction

The need for protection against loss or gain of water vapour by foods, particularly when frozen or dehydrated, is generally recognized in the food industry today. For this reason, a limited study of the water-vapour resistance of packaging materials has already been made in this laboratory (3). It was felt desirable to continue this work and evaluate thoroughly the effectiveness of the more important types of flexible barriers available in Canada.

During the course of preliminary studies (3), the question of the relative effectiveness of carton liners and overwraps was raised. In addition, no precise information was available to permit selection between the foregoing methods and wax-dipping as a means of providing protection against water-vapour penetration. Hence, prior to investigating individual materials an experiment was designed to evaluate the resistance to rough handling of packages made by these various methods.

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Relative Fragility of Water-vapour Barriers when Applied by Various Methods

Materials and Methods

The packaging materials selected for use in this portion of the investigation were: 450 M.S.Y.T. "Cellophane," Reynolds' Metal A-10 (a lamination of kraft to metal foil to Cellophane), and Darex Wax P-16 (a commercial dipping wax). These materials were used to prepare liners and overwraps or for wax-dipping 12 cartons, 4 by $2\frac{3}{4}$ by $1\frac{1}{8}$ in. (opening end $2\frac{3}{4}$ by $1\frac{1}{8}$ in.). These cartons were of the regular flat folding style with full overlapping long flaps and were made of 0.020 in. chipboard. The packages were filled with sawdust, closed, and packed in a master container which accommodated three layers each containing six by four or 24 upright packages. The master container was $12\frac{1}{4}$ by $11\frac{1}{8}$ by $12\frac{1}{4}$ in. high (opening end $12\frac{1}{4}$ by $11\frac{1}{8}$ in.), and was made of regular B-flute, utilizing 0 016 - 0 009 - 0 016 Fourdrinier kraft liner and corrugations. Of 72 cartons in each master container, a set of 12 was under study and were packed in fixed positions (Fig. 1), the remaining space being filled with dummy packages containing sawdust.

Three experimental conditions of rough handling were investigated. The first involved cooling the packages to minus 40° F, and then dropping them 20 times through a distance of three feet to a cement floor (five falls on each of the four upright edges of the master container). The second treatment followed the same procedure at room temperature (approximately 75° F.). The third permitted a single fall of about 70 ft. on to cement (temperature of packages, approximately 75° F). After treatment the packages were opened and the water-vapour barriers were examined visually for fractures, and for pin holes.

Results

The results of the visual examination of the packaging materials are given in Table I, and the data are summarized on a per cent basis in Table II. Of the materials used, Cellophane did not develop pin holes as easily as did Reynolds' Metal A-10, but it was more prone to fracture. Over-all, Cellophane appeared slightly more flexible than Reynolds' Metal, but this was not borne out by subsequent work, as will be shown later.

Reduction in temperature greatly increased the fragility of barriers when roughly handled. In addition, a number of short falls, a condition more likely to be met in ordinary commercial handling and transport, caused greater damage to the water-vapour barrier than a single fall from a much greater height.

Under the handling conditions described, wax-dipping was generally less desirable than either of the other two methods of providing water-vapour protection. While Darex P-16 may not be the most flexible wax obtainable,

TABLE I

FRAGILITY OF WATER-VAPOUR BARRIERS WHEN ROUGHLY HANDLED

Method of providing water-vapour barrier	Method of handling	Number of barriers		
		Unbroken	With pinholes	Fractured
<i>Cartons with liners</i>				
450 M.S.Y.T. Cellophane } Reynolds' Metal A-10 }	3 ft. drop, 20 falls at -40° F.	{ 2 0	2 10	8 2
450 M.S.Y.T. Cellophane } Reynolds' Metal A-10 }	3 ft. drop, 20 falls at approx. 75° F.	{ 7 3	2 9	3 0
450 M.S.Y.T. Cellophane } Reynolds' Metal A-10 }	70 ft. drop (approx.), one fall at approx. 75° F.	{ 11 9	1 1	0 2
<i>Overwrapped cartons</i>				
450 M.S.Y.T. Cellophane } Reynolds' Metal A-10 }	3 ft. drop, 20 falls at -40° F.	{ 0 0	0 2	12 10
450 M.S.Y.T. Cellophane } Reynolds' Metal A-10 }	3 ft. drop, 20 falls at approx. 75° F.	{ 4 0	4 9	4 3
450 M.S.Y.T. Cellophane } Reynolds' Metal A-10 }	70 ft. drop (approx.), one fall at approx. 75° F.	{ 8 6	1 3	3 3
<i>Wax-dipped cartons</i>				
Darex P-16	3 ft. drop, 20 falls at -40° F.	0	0	12
Darex P-16	3 ft. drop, 20 falls at approx 75° F.	0	0	12
Darex P-16	70 ft. drop (approx.), one fall at approx. 75° F.	0	0	12

the results are nevertheless indicative of what may be expected from this type of packaging using currently available waxes. It has been observed elsewhere (2) that rough handling caused an appreciable increase in the moisture gain of wax-dipped packages of dehydrated pork.

The most desirable method of providing water-vapour protection was a liner inside the carton (Table II). This method cannot be used, however, when the contents of the package are of such a nature as to effect possible rupture of the inner barrier. In such circumstances overwrapped cartons seemed to be more desirable. Subsequent work has shown, however, that the best method of packaging such materials is by the use of the container-barrier-container method (1). This consists of placing the product in a light carton, applying the water-vapour barrier, and placing the whole in another snugly fitting carton.

TABLE II

SUMMARY OF FACTORS AFFECTING THE FRAGILITY OF WATER-VAPOUR BARRIERS
CONSIDERED OVER OTHER CONDITIONS

Factor	Per cent of barriers		
	Unbroken	With pinholes	Fractured
<i>Method of providing barrier</i>			
Liner	44	35	21
Overwrap	25	26	49
Wax-dip	0	0	100
<i>Material</i>			
Cellophane	44	14	42
Reynolds' Metal A-10	25	47	28
Darex P-16	0	0	100
<i>Method of handling*</i>			
3 ft. drop, 20 falls at -40° F.	4	29	67
3 ft. drop, 20 falls at approx. 75° F.	29	50	21
70 ft. drop (approx.), single fall at approx. 75° F.	71	12	17

*Summary confined to liners and overwraps only.

Water-vapour Transmission of Packaging Materials

Materials and Methods

Earlier work has indicated that the most satisfactory evaluation of the water-vapour resistance of packaging materials was obtained after fabrication into packages (3). Moreover, the experiment described above showed that the most desirable method of using a water-vapour barrier was as a liner inside the package. Hence, in the present work, all barriers (with one exception as noted below) were fabricated into carton liners.

The materials tested were different grades and plies of Cellophane, and various combinations of metal foil, scrim (a material similar to cheesecloth), kraft paper, glassine, Cellophane, Pliofilm, cellulose acetate, and vinylite. In addition, some of the materials were tested after waxing. Detailed descriptions of individual materials are given in Tables III, IV and V.

The materials were fabricated into pouch type liners, having outside dimensions of $5\frac{3}{8}$ by $6\frac{3}{4}$ in. high and inside dimensions of $4\frac{3}{8}$ by $6\frac{1}{4}$ in. high, suitable for use inside the chipboard carton which has already been described. The liners were opened, inserted into the cartons and partially filled with sawdust; then 73.5 gm. of anhydrous calcium chloride in a perforated P.T. Cellophane bag was added; this bag was surrounded by sawdust and the remainder of the liner was filled with sawdust. The liner and cartons were then sealed, sodium silicate glue being used to make the carton closure.

The one exception to the above procedure was the material composed of scrim laminated to M.S.A.T. Cellophane, both sides being waxed with micro-

crystalline wax. This material was designed for overwrapping, and so was applied in this way. A double fold was made at the side seam, and completed packages were dipped in microcrystalline wax held at 170° F.

Five tests were done on each packaging material, six completed packages being used for each test. Six packages without calcium chloride and sawdust were used as a means of estimating the sorption of water vapour by the packaging materials. To estimate the sorption by the material used as an overwrap, a set of dummy packages of the same size as the chipboard cartons used was made, using metal in place of the chipboard box, to eliminate all absorbent material beneath the barrier.

One set of six packages was placed in a cabinet operating at 95° F. and 100% relative humidity (high humidity cabinet, vapour-pressure differential approximately 42 mm. of mercury). Another set was placed in an alternating cabinet which operated at 80° F. and 100% relative humidity (vapour-pressure differential approximately 26 mm. of mercury) for 12 hr., and 120° F., 55% relative humidity (vapour-pressure differential approximately 48 mm. of mercury), for the remaining 12 hr. of the day. The latter test was designed to give packages an opportunity to breathe.

The remaining three sets of packages were subjected to various treatments before the water-vapour penetration was determined in the high humidity cabinet. One set was stored at 140° F. (relative humidity about 6%) for one month, to evaluate the effect on the barrier of storage under hot dry conditions. To simulate very severe conditions of handling and transport, one set was cooled to minus 40° F. and dropped three feet, 20 times (five falls on each upright edge of the master container). After cooling to minus 40° F. the remaining set was subjected to a vacuum of 20 in. of mercury for two hours, to reproduce conditions encountered in air transport. Dropping and low pressure tests were performed using a master carton, which has already been described. The arrangement of test packages in the master container is shown in Fig. 1. It will be noted that, in the dropping test, two of the packages were buried in the interior of the master carton, being surrounded by dummy packages, while the remaining four were placed at the edges of the master carton.

To determine moisture gain, packages, as individual units, were weighed before insertion in the cabinets and at weekly intervals for four weeks.

Since packages are not normally stored in an atmosphere of 95° F., 100% relative humidity, it was desirable to be able to interpret water-vapour transmission rates in terms of temperate room conditions. Hence, the transmission of three materials (Reynolds' Metal A-10, 300 M.S.A.T. Cellophane, and 300 M.S.T. Cellophane wax-coated 40 lb. per ream) was determined under the conditions existing in the laboratory, as well as in the high humidity cabinets. Packages were made up as previously described, and weighed at weekly intervals from August 1, 1945, to February 1, 1946.

Results

Water-vapour transmission rates are shown in Tables III, IV and V. To obtain these values, the weight of water vapour sorbed by the packaging materials (empty packages) at a given time was subtracted from the total increase in weight of the test packages, weighed at the same time. The slope

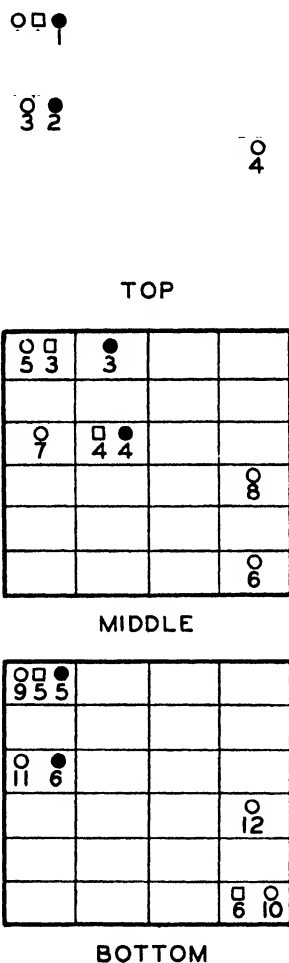


FIG. 1. Packages in master container, arranged for handling trials (packages under study marked, all others dummies)

- Dropping test, methods of application trials.
- Low pressure test.
- Dropping test, material trials

of the line showing weight gain per week of the package contents over a period of one month was then calculated assuming a straight line relation.

The average standard error for the transmission rates, except those obtained after the dropping test, was 0.24, hence rates must differ by 0.48 gm. per

TABLE III

SUMMARY OF WEIGHT CHANGES IN PACKAGES UTILIZING CELLOPHANE AS A LINER

Material	Thick- nesses	Seal	Sorption (gm.) by packaging materials held in high humidity cabinet	Water-vapour transmission (gm./week), high humidity cabinet, after treatments as follows:				Water- vapour trans- mission (gm./ (week), alternat- ing cabinet
				Untreated	One month at 140° F.	Subjection to low temp and low pressure	Dropping* 20 times at -40° F	
300 M.S.T.	Duplex	Crimp	1 84	0 89	10 78	1 66	6 82	1 44
300 M.S.A.T.	Duplex	Crimp	2 68	0 79	3 23	1 31	2 32	0 99
300 M.S.Y.T.	Duplex	Crimp	2 50	0 48	1 22	0 86	0 97	0 65
300 M.S.A.T.	Single	Crimp	1 44	1 46	8 83	2 75	4 92	1 30
300 M.S.A.T.	Duplex	Crimp	2 68	0 79	3.23	1 31	2 32	0 99
300 M.S.A.T.	Triplex	Crimp	3 37	0 25	0 62	0 82	2 17	0 78
300 M.S.A.T.	Duplex	Flat	2 96	0 31	5 50	0 96	2 87	0 83
300 M.S.A.T.	Duplex	Crimp	2 68	0 79	3 23	1 31	2 32	0 99

* Average for only two packages (see Table VI).

week to be significantly different. As shown by Table VI, when most of the materials tested were subjected to dropping at $-40^{\circ}\text{F}.$, the four packages against the edges of the master container were fractured. For this reason, the water-vapour transmission rates obtained after the dropping were calculated from the gains of only the two packages buried in the interior of the master carton, and the standard error for these rates was larger, being 0.79. Keeping the above limits of accuracy in mind, it is possible to compare the protection offered by different materials under the conditions used.

Little difference was noted between the water-vapour transmission of untreated packages in the alternating and constant temperature cabinets, but any treatment simulating storage or transport markedly increased the rate of moisture gain (Tables III, IV, and V). After subjection to low temperature and pressure, the majority of packages showed a substantial increase in water-vapour penetration; however, the increase was no greater than that caused by the other handling tests, and no fractures in packaging material resulted from this treatment. This indicates that air transport should not damage packages extensively, provided air volumes inside the barriers are kept to a minimum.

When Cellophane was considered, it was apparent that M.S.Y.T. stock provided greater protection than M.S.A.T., which in turn was better than M.S.T. (Table III). The use of triplex bags gave maximum resistance to water vapour. A flat seal appeared to be generally more desirable than a crimp seal, although the latter was superior for excessively high storage temperatures.

TABLE IV

SUMMARY OF WEIGHT CHANGES IN PACKAGINGS USING PLAIN OR WAX-COATED MATERIALS AS LINERS

Stock	Processing	Heat sealed	Sorption (gm) by packaging materials held in high humidity cabinet	Water vapour transmission (gm/week) high humidity cabinet after treatment as follows				Water vapour transmission (gm/week) alternating cabinet
				Untreated	One month at 140° F	Subjection to low temp and low press	Dropping* 20 times at -40° F	
40 lb kraft	Wax impregnated	(Adhesive sealed)	3.38	14.0‡	7.32‡	13.2‡	17.8‡	6.16
40 lb wet strength kraft	Wax coated†† 40 lb/ream	Flat	7.58	1.42	1.77	1.86	3.79	2.83
25 lb bleached glassine	Thermoplastic coated one side	Flat	1.94	4.97	†	6.98	15.66	4.61
25 lb bleached glassine	Wax coated†† 40 lb/ream	Flat	8.46	0.84§	1.19	1.25§	†	2.06
300 M S T Cellophane	None	Flat	2.28	1.85	33.0‡	2.41	10.06	1.50
300 M S T Cellophane	Wax coated†† 40 lb/ream	Flat	8.50	0.79	0.31	3.02	†	0.80
Laminated 300 M S T Cellophane	None	Flat	4.10	0.59	0.08	0.46§	1.76	0.66
Laminated 300 M S T Cellophane	Wax coated†† 40 lb/ream	Flat	3.57	0.24	0.06	0.08	0.89	0.04
Phofilm	None	Flat	1.43	1.18	1.27	1.35	2.03	1.16
Scrim laminated to M S A T Cellophane waxed both sides	Applied as overwrap Package wax dipped							
	(a) Scrim side in	—	0.18	0.17	0.39	0.13	0.19	0.11
	(b) Scrim side out	—	0.75	0.02	0.22	0.39	0.17	0.14

* Average for only two packages (See Table VI)

‡ Averages for five packages only—one failure under these conditions

† Complete failure under these conditions

†† Flexible wax composition

‡ Measurement for first two weeks only, complete failure

Wax-coating (40 lb per ream) various base stocks reduced the moisture penetration to less than one-half, but the transmission of all stocks was not reduced to a common value, i.e., the more dense the base stock the lower the water-vapour transmission after waxing (Table IV). Wax-coating Cellophane and glassine reduced the moisture gain after ageing at 140° F, but did little to increase resistance to fracture at low temperature (Tables IV and VI). It will be noted that the material that was applied as an overwrap withstood dropping slightly better than other waxed materials. This was due to the fact that the wax on this material was more flexible than that on the coated materials, and

to the strength imparted to it by the scrim incorporated into the sheet. It must be borne in mind, however, that the transmissions reported after drop-

TABLE V

SUMMARY OF WEIGHT CHANGES IN PACKAGES USING LAMINATED MATERIALS AS LINERS

Stock	Laminated to (wax laminated unless otherwise stated)	Heat sealed	Sorption (gm) by packaging materials held in high humidity cabinet	Water vapour transmission (gm/week), high humidity cabinet after treatment as follows				Water- vapour trans- mission, (gm / week) alternat- ing cabinet
				Un- treated	One month at 140° F.	Sub- jection to low temp and low press	Drop- ping* 20 times at -40° F.	
Scrim (Reynolds A-50)	Kraft and alloyed lead foil with butvar coating (asphalt lam)	Flat and reinforced with cellulose tape	1.79	0.02	0.25	0.00	0.60	0.04
25 lb kraft	25 lb kraft thermo- plastic coated one side	Flat	2.04	8.92	29.26†	17.96†	15.55†	4.92
25 lb kraft	25 lb glassine thermo- plastic coated on glassine	Flat	2.40	2.36	10.90‡	2.64	3.63	3.77††
White kraft	300 M S T Cello- phane	Crimp	3.92	0.80	0.46	1.13	5.48	0.43
25 lb kraft	300 M S A I Cello- phane	Flat	3.50	0.80	1.30	1.40	2.83	0.79
25 lb kraft	Cellulose acetate thermoplastic coated on acetate side	Flat	2.24	1.62	9.73	4.78	6.58	1.42
25 lb kraft (Reynolds A 15)	Alloyed lead foil (asphalt lam) thermoplastic coat on foil	Flat	0.85	0.24	0.30	0.34	1.20	0.22
40 lb kraft (Reynolds A-10)	Alloyed lead foil and Cellophane (asphalt lam)	Flat	2.15	0.00	0.13	0.10	0.97	0.13
25 lb glassine	25 lb glassine thermoplastic coated one side	Flat	2.08	1.24	1.14	1.59	1.92	3.78
25 lb glassine	300 M S T Cello- phane	Crimp	1.03	0.77	0.21	0.99	1.95	0.49
300 M S T Cellophane	300 M S I Cellophane	Flat	4.10	0.59	0.08	0.46§	1.76	0.66
300 M S T Cellophane	Aluminum foil	Crimp	1.43	0.05	0.37	0.35	1.43	0.19
Vinylite	Both sides of aluminum foil	Flat	2.06	0.00	0.00	0.00	0.00	0.00

* Average for only two packages (See Table VI).

† Complete failure under these conditions.

‡ Measurements for first three weeks only, complete failure.

§ Average for five packages only, one failure under the conditions.

†† Average for four packages only, two failures under these conditions.

TABLE VI

ABILITY OF PACKAGES TO WITHSTAND 20 DROPS OF THREE FEET EACH AT -40° F. (-40° C.)

Stock	Processing	Packages unbroken—* (six tested)	Packages with water-vapour transmission comparable to that of interior packages
Scrim (Reynolds' A-50)	Laminated to kraft and foil with butvar coating	6	4
40 lb. kraft	Wax-impregnated	6	0
White kraft	Laminated to 300 M.S.T. Cellophane	6	1
Metal foil	Laminated to 300 M.S.T. Cellophane	6	0
Scrim laminated to M.S.A.T. Cellophane	Applied as overwrap. Package wax dipped. (a) Scrim side in	6	1
waxed both sides	(b) Scrim side out	6	0
Kraft (Reynolds' A-10)	Laminated to metal foil and Cellophane	5	0
25 lb. kraft (Reynolds' A-15)	Laminated to metal foil with thermoplastic coating	5	0
25 lb. kraft	Laminated to cellulose acetate	5	1
Vinylite	Laminated to both sides of aluminum foil	4	2
40 lb. kraft (wet strength)	Wax-coated 40 lb /ream	3	0
25 lb. kraft	Laminated to 25 lb. glassine	3	0
25 lb. kraft	Laminated to 300 M.S.A.T. Cellophane	3	0
300 M.S.T. Cellophane	Laminated to 300 M.S.T. Cellophane	3	1

* All other types had only two interior packages unbroken (see Tables III, IV, and V).

ping were based on the two interior packages only, and must be considered in conjunction with Table VI.

Laminated materials having metal foil as one layer provided greater protection than any other materials (Table V). The results also show that laminating two stocks produced marked reduction in the water-vapour transmission typical of either base sheet when used alone. The lamination of vinylite to metal foil illustrates that it is possible to produce a very water-vapour resistant package by combining a material that is not water-vapour resistant with one that is. Vinylite is not considered water-vapour proof and was utilized in this combination to provide strength and heat sealing properties.

Table VI shows the number of packages remaining unfractured after the dropping test, and indicates that the great majority of materials tested require protection from such treatment. Laminations utilizing scrim or kraft were less likely to fracture when roughly handled, but absence of a visible break did not necessarily mean that the water-vapour transmission rate had not increased considerably.

Under room conditions, the water-vapour transmission rates of Reynolds' Metal A-10, 300 M.S.A.T. Cellophane, and 300 M.S.T. Cellophane wax-coated 40 lb. per ream were 0.00, 0.20, and 0.11 gm. per week, respectively. The foil barrier had an undetectable transmission rate in the high humidity cabinet (untreated packages, Table V), and this was also true under room conditions. However, for 300 M.S.A.T. Cellophane and for wax-coated Cellophane, the ratio of the water-vapour transmission in the high humidity cabinet to the transmission under room conditions was 7.3 and 7.2, respectively.

Acknowledgments

The authors wish to express their gratitude to the many commercial firms who contributed materials during the course of this work, and to Messrs. A. J. Cameron and R. C. Wright of Canadian Industries Ltd., and Dr. A. H. Woodcock of E. S. & A. Robinson (Canada) Ltd., for their kind advice.

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DRIED WHOLE EGG POWDER

XXII. SOME FACTORS AFFECTING THE PRODUCTION AND INITIAL QUALITY OF DRIED SUGAR-AND-EGG MIXTURES¹

BY JESSE A. PEARCE², J. BROOKS³, AND H. TESSIER⁴

Abstract

Sugar egg powder was produced under a variety of conditions in a laboratory spray drier and in two commercial driers. A product prepared at inlet temperatures below 270° F. and outlet temperatures below 150° F. was the most suitable for baking purposes and was generally the best when assessed by measurements of fluorescence, potassium chloride value, and pH. Powder of particle size small enough to pass an 80 mesh screen (U.S. Bureau of Standards) appeared to have better baking properties than coarser material. Trials with nozzles of various sizes indicated that the best product was prepared using small nozzles. Sucrose syrup or solid sucrose, with fresh or frozen egg, all produced powders of similar initial quality.

Introduction

The addition of sucrose sugar to liquid egg before drying is known to provide protection during heat treatment (2) and storage of the dried product (1, 3). The product is much more suitable than plain egg powder for use in baked goods (2). However, no information was available about the best conditions for producing this material. Since it is expected that Canada will produce about 20½ million pounds of dried sugar-egg powder during 1946, it was believed desirable to examine certain factors in the processing procedure that may affect the quality.

Materials and Methods

The liquid from fresh, Grade A, shell eggs, except as noted in Fig. 1, was used in operations on the laboratory cone-type drier (11), while liquid from frozen egg was used for all work on two commercial cone-type driers, except as noted in Table II.

Rate of production at specific drying conditions for some of the work done in the commercial plants is given in Table I. The effect of air temperature and nozzle diameter on the rate of powder production can be estimated from Table I and the data in the other tables and Fig. 1.

The quality of the powder was determined by measurement of the moisture content (9), fluorescence value (5), potassium chloride value (9), pH (4), baking volume (6), foaming volume (6), and foam stability. In addition,

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TABLE I

DRYING CONDITIONS AND RATE OF SUGAR-EGG PRODUCTION FOR SOME OF THE WORK DONE IN THE COMMERCIAL PLANTS

Plant	Inlet temperature, ° F.	Outlet temperature, ° F.	Nozzle diameter, in.	Pump pressure, p.s.i.	Production, lb./hr.
1	285	155	0.0635	4300	700
2*	240	155	0.0700	4400	700

* Uses preheater on egg just before it goes to spray nozzle in drier.

some samples were subjected to a sieve analysis to obtain fractions of different particle sizes. The procedures for determining baking volume and foaming volume were unsatisfactory and were modified as noted below.

To prepare sponge cakes for the determination of baking volume, all the materials used were brought to a temperature of 80° F. and all mixing was done in a room at 80° F. and 65% relative humidity. The procedure was as follows.

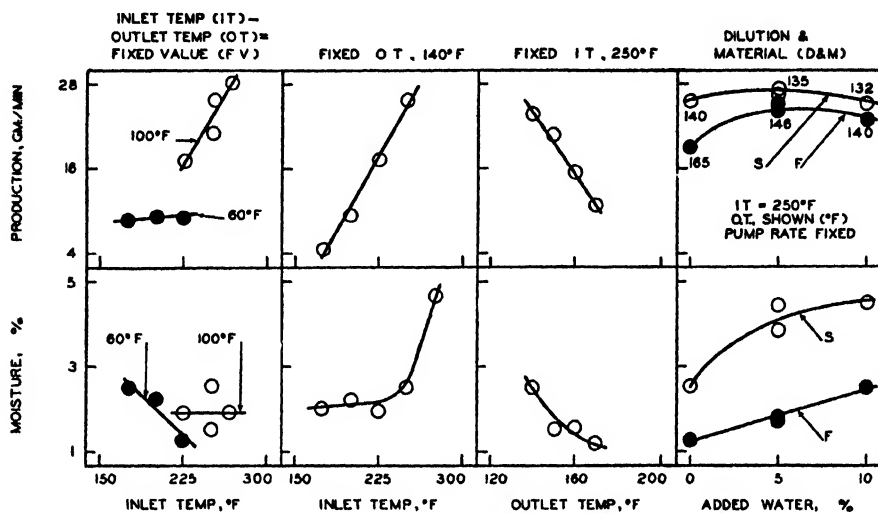
Sugar-egg powder (9 gm.) was mixed, by hand, with 17 gm. of commercial, powdered sugar (sucrose) in the small bowl of a Mixmaster. A portion of a measured volume of 19 ml. of tap water was added and mixed into a paste with the sugar and powder. The remainder of the water was added and mixed with the paste until a homogeneous liquid was obtained. The beaters of the Mixmaster were then lowered into the bowl so they just touched the bottom and were allowed to whip the mixture for 10 min. while operating at No. 10 speed. After five minutes' beating, the bowl was turned by hand through 90°, but no other motion of the bowl was permitted. Small portions of a 20 gm. quantity of a standardized super-cake flour were sprinkled over the surface and each portion was carefully mixed in with a rubber spatula in such a manner that the foam was disturbed as little as possible. The batter was then carefully scraped from the beaters and bowl and transferred to an ungreased pan, which was immediately inserted into an oven (400° F.) and baked for 15 min. After baking, the sponge and pan were inverted and allowed to stand in the conditioned room overnight. The next day, the volume of the cake was measured. The standard deviation of this volume-measuring technique was 2.4 ml.

It was observed that two different technicians produced cakes with an average difference in volume of 12 ml., therefore, most of the baking was done by one person only. For this person, three cakes were necessary to show a significant difference of 10 ml. in the baking volume of the powders. Therefore, all values shown are the average of volume measurements on three cakes. No significant day-to-day differences in cake volume were observed.

To determine foaming volume the requirements for conditioning and mixing the materials were the same as for the baking test. Otherwise, the

quantities of material and the procedure described elsewhere were used (2). Foam stability was evaluated by inverting the graduate cylinder, in which foaming volume was measured. The time required for the foam to begin dripping from the cylinder was determined.

LABORATORY DRIER



COMMERCIAL DRIERS

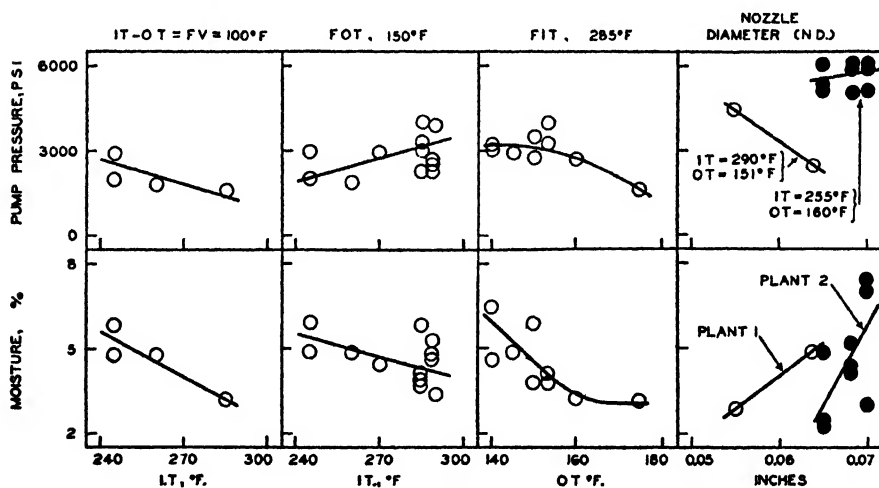


FIG 1 The effect of drying conditions on the moisture content and rate of production (shown as pump pressures in commercial driers) of sugar-egg powder.

Laboratory drier ○, powder from fresh shell eggs (S), ●, powder from frozen liquid eggs (F)

Commercial drier: ○, operations at Plant 1, ●, operations at Plant 2.

Particle size separations were done using screens of 16, 35, 65, 100, 200, and 325 mesh (U.S. Bureau of Standards). Previous examination indicated that one hour on a Ro-tap shaker was the most desirable sieving time, and this sieving period was used throughout. However, it has since become apparent that even this time did not provide complete separation of particles of different size (8).

Results

Laboratory Drier

The use of low drying temperatures reduced the rate of production and resulted in increased moisture content in the powder (Fig. 1); this corroborated the results of a previous study on plain egg powder (10). However, powder produced at an inlet temperature of 280° F. and an outlet temperature of 140° F. had an unexpectedly high moisture content. This may be attributable to the low volume of air passing through this drier and the high liquid flow rates necessary to maintain the low outlet temperature. Under these conditions, adequate removal of the water vapour was not possible.

As with plain egg powder (10), the present work showed that the best product, as assessed by all quality measures, was produced at the lowest temperatures (Fig. 2). One anomalous result was noted in the studies using fixed outlet temperatures. Materials produced at an outlet temperature of 150° F. had lower fluorescence values than material produced at 140° or 160° F. These products also had higher foaming volumes than material produced at 140° F. In general, the results indicated that, for this drier, good quality sugar-egg powder could be produced at an inlet temperature of 270° F. and that outlet temperatures of about 140° to 150° F. were satisfactory.

For a fixed pump rate a smaller amount of powder was produced from frozen melange than from fresh liquid egg (Fig. 1). This was attributable to the greater viscosity characteristic of stored, frozen, liquid egg. (It is possible that this high viscosity might be reduced by the addition of sugar to the liquid egg before freezing.) The moisture content was lower owing to the higher outlet temperatures associated with the lower throughput. However, by appropriate dilution, it was possible to prepare material from frozen melange that was similar in quality to that prepared from fresh eggs. As might be expected, at a fixed pump rate, dilution reduced the outlet temperature, with a corresponding increase in powder quality and moisture content.

Several additional factors were examined using the laboratory drier (Table II). These results showed that no significant difference resulted from the use of solid sugar or sucrose syrup, or from the sugars currently available in Canada and likely to be used by the various producers. Rapid beating of the sugar and liquid egg before drying increased the fluorescence value and decreased the potassium chloride value although it had no significant effect on baking quality.

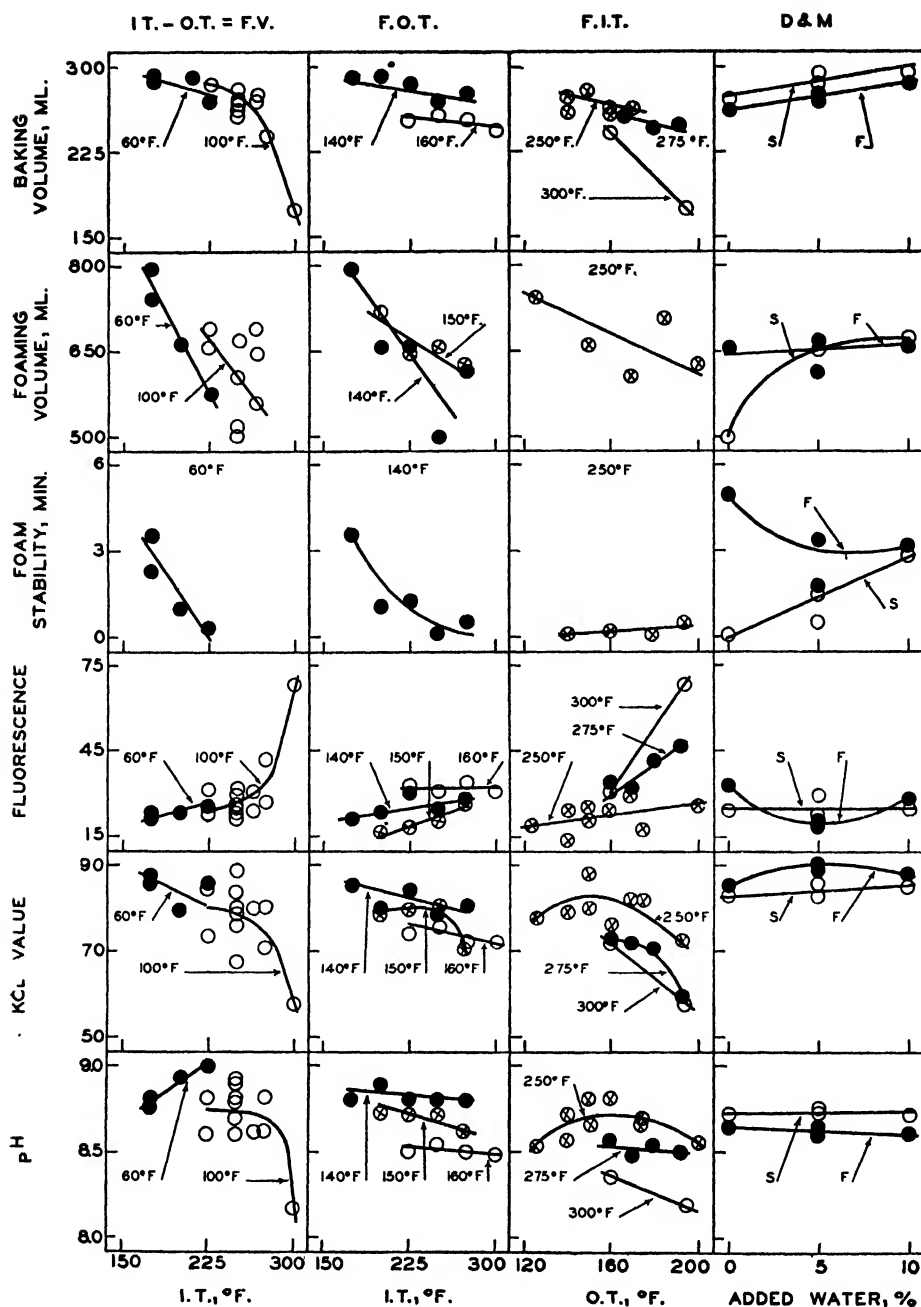


FIG. 2. The effect of drying conditions on the quality of sugar-egg powder produced on laboratory drier (for abbreviations, see Fig. 1): ○, powder from fresh shell eggs (S); ●, powder from frozen liquid eggs (F). The average baking volume for sponges prepared from shell eggs was 286 ml.

TABLE II

EFFECT OF MISCELLANEOUS ITEMS ON POWDER PRODUCTION AND POWDER QUALITY—LABORATORY DRIER

Item	Number of trials	Inlet temp., ° F.	Outlet temp., ° F.	Powder production, gm./min	Powder quality						
					Moisture, %	Baking volume, ml.	Foaming volume, ml.	Foam stability, min.	Fluorescence value	Potassium chloride value	pH

Sugar vs. syrup (about 55% sucrose)

Set 1												
Sugar	9	250	150	18.8	2.8	—	662	—	22.1	76.7	8.6	
Syrup	9	250	150	14.2	4.0	—	682	—	21.1	78.6	8.6	
Set 2												
Sugar	3	225	160	—	—	273	—	—	34.8	78.8	8.7	
Syrup	3	225	160	—	—	265	—	—	34.6	77.1	8.5	

Effect of sugar from different areas

Alta. (beet)	3	250	140	—	—	284	670	2.5	17.7	81.2	8.8	
Man. (beet)	3	250	140	—	—	286	687	2.5	18.3	83.5	8.8	
Ont. (cane)	3	250	140	—	—	286	710	4.0	18.2	81.1	8.8	

Effect of rate of stirring egg and sugar before drying

Fast (causes foaming)	2	250	140	25.8	2.78	280	633	1.5	26.1	84.8	8.8	
Slow (no foaming)	2	250	140	25.8	3.05	276	656	1.9	21.8	88.0	8.6	

Commercial Driers

For the commercial driers, as for the laboratory drier, it was apparent that reduction in the inlet temperature, with a fixed outlet temperature, decreased production (estimated from pump pressure changes noted in Fig. 1) and resulted in a product with increased moisture content. Reducing the diameter of the spray nozzle lowered the moisture content, but did not affect production if the pump pressure was increased.

By all quality criteria, except foam stability and pH, the best product was produced at the lowest drying temperatures (Fig. 3). The foam stability measurement gave irregular results but this measurement was believed to be of less importance than baking volume. No explanation can be offered for the exceptionally high pH values observed for material produced at inlet temperatures higher than 285° F. for Plant 1 and 280° F. for Plant 2. In general, inlet temperatures of 270° F. and lower were most satisfactory. For Plant 1, an outlet temperature of 150° F. or lower was most desirable. Because of

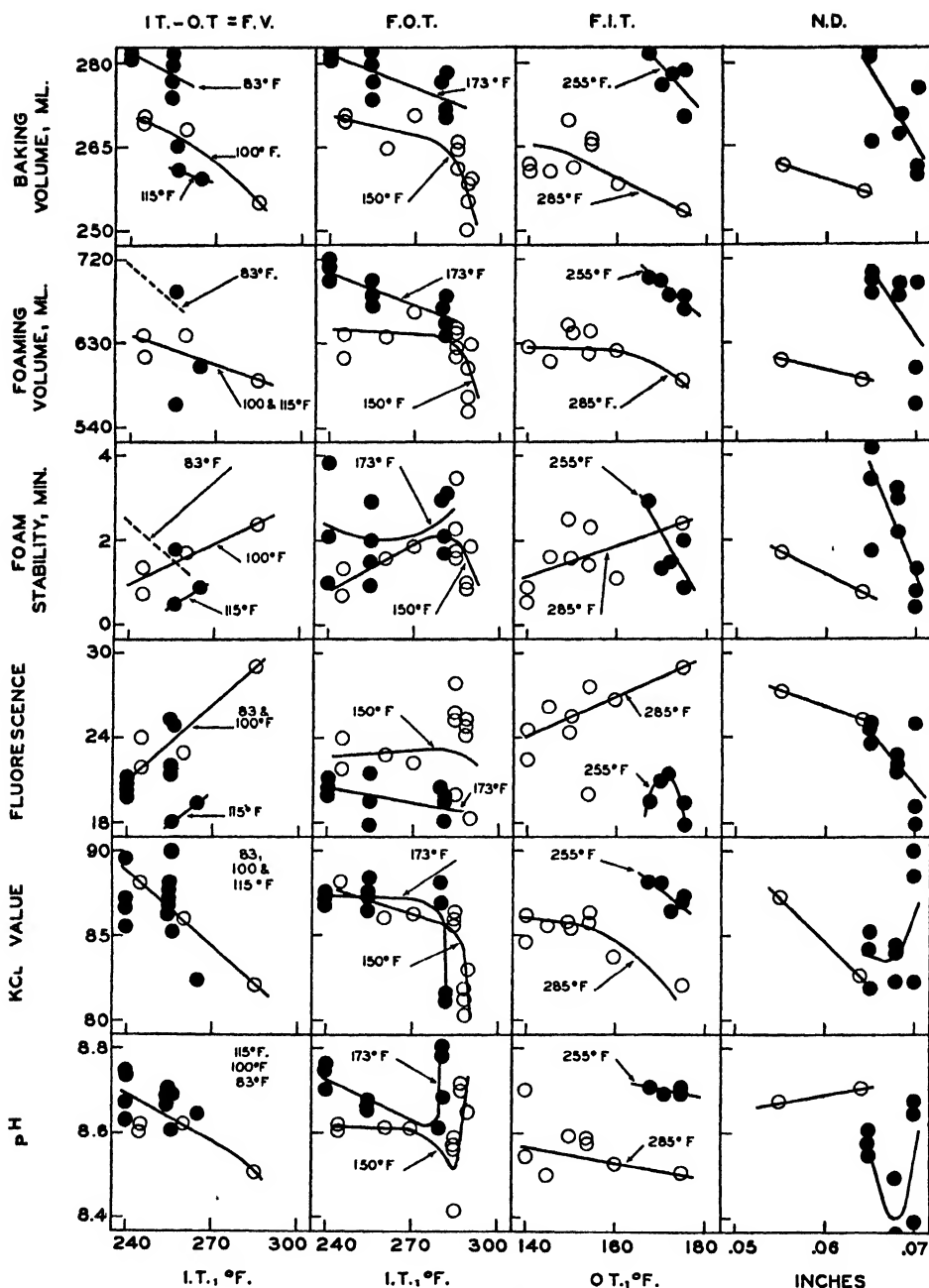


FIG. 3. The effect of drying conditions on the quality of sugar-egg powder produced on commercial driers (for abbreviations, see Fig. 1): ○, operations at Plant 1; ●, operations at Plant 2; dotted lines, trials at Plant 2, points omitted to avoid confusion. All studies shown in the first three columns done at nozzle diameters of 0.064 in. and 0.070 in. Plants 1 and 2, respectively. The average baking volume for sponges prepared from shell eggs was 286 ml.

the limited range studied it was difficult to evaluate the most desirable outlet temperatures for Plant 2, but, again, the indications were that the lowest temperatures were the most desirable.

The size of the spray nozzle used was important. Results from both plants showed that the smaller the nozzle diameter the better the baking quality of the product and the lower the moisture content of the powder, but the fluorescence values were slightly increased. Nevertheless, the powders produced would meet the requirements of the tentative specification for Grade A sugar-egg powder (7). Further trials in Plant 2, using a multiple nozzle (three openings, 0.055 in.), gave products with baking volumes of 289 and 291 ml.

Baking tests were also done on fractions sieved from the various powders (Table III). These results supported the foregoing evidence and indicated that, for best baking quality, the powder should be fine enough to pass an 80 mesh screen.

TABLE III

THE EFFECT OF PARTICLE SIZE ON THE BAKING QUALITY OF SUGAR-EGG POWDER

Baking volume of whole sample, ml.	Baking volume (ml.) of sieved portions falling between the following sieve sizes (U.S. Bureau of Standards):					
	16-35	35-65	65-80	80-100	100-200	200-325
283	—	—	262	273	279	—
282	—	—	—	—	276	279
269	—	274	282	—	272	—
266	—	267	—	264	272	—
266	—	275	265	—	257	—
265	—	259	254	—	265	—
265	—	228	250	—	252	254
264	—	268	272	—	271	—
259	—	268	261	275	269	—
259	248	254	256	—	257	—
Average	248	262	263	271	263	266

Several additional factors were examined (Table IV). The increased temperature difference necessary when producing powder in wet weather caused a slight increase in fluorescence value and a slight decrease in potassium chloride and foaming volume values. The temperature of the powder at the time of packing had no significant effect on the baking quality of the product. Differences in powders produced from diluted liquid egg (fresh, shell), mixtures of frozen and shell egg liquids, and frozen egg were not significant. Powders of similar quality were produced by the addition of sugar in either solid or liquid form.

Discussion

The results obtained using either the laboratory or commercial driers showed that sugar-egg powder with excellent baking properties can be produced at inlet temperatures of 270° F. or lower and at outlet temperatures

TABLE IV

EFFECT OF MISCELLANEOUS ITEMS ON POWDER PRODUCTION AND POWDER QUALITY—COMMERCIAL DRILRS

Item	Number of trials	Inlet temp ° F	Outlet temp ° F	Powder production gm/min	Powder quality						
					Moisture %	Baking volume ml	Foaming volume ml	Foam stability, min.	Fluorescence value	Potassium chloride value	pH
Dry weather	9	285	255	3244	3.92	262	632	2.2	22.7	86.2	8.6
Wet weather	8	290	250	3098	4.67	260	593	1.2	24.1	84.3	8.6

Powder packaged at average temperature of 78° F

Plant 1	2	289	152	3170	4.05	254	610	1.4	21.7	83.6	8.7
Plant 2	1	255	162	6000	4.12	267	695	3.0	21.6	84.1	8.4
Average					4.07	258	638	1.9	21.7	83.8	8.6

Powder packaged at average temperature of 106° F

Plant 1	2	288	151	2400	5.23	256	564	1.0	24.5	82.5	8.7
Plant 2	1	255	162	6000	4.33	270	695	3.5	22.9	84.8	8.2
Average					4.93	261	604	1.8	24.0	83.3	8.5

Frozen vs fresh shell liquid, and dilution

Shell (undiluted)	3	285	152	3300	3.22	260	627	2.0	21.4	86.8	8.6
Shell plus 7% water	1	285	154	3300	3.81	260	617	2.5	25.8	87.2	8.6
1 part shell and 3 parts frozen	1	285	153	3000	4.01	264	628	3.5	25.5	86.2	8.4
Frozen plus 7% water	4	285	151	3680	3.86	266	616	2.0	23.0	86.8	8.6

Sugar vs. syrup (about 55% sucrose)

Plant 1											
Sugar	3	285	154	3350	4.02	265	640	2.3	20.2	86.8	8.6
Syrup	3	285	155	3300	3.76	257	611	2.2	22.7	86.0	8.6
Plant 2											
Sugar	1	257	155	6000	4.94	266	685	1.8	24.9	85.2	8.6
Syrup	1	255	160	5300	2.52	283	688	3.4	25.0	83.8	8.6

of 150° F. or lower. These conditions permitted fairly rapid production and, when small nozzles were used, produced powder with less than 3.0% moisture (2). It is also of interest to note that in the commercial trials the plant using a preheater for the liquid egg produced powder with better baking quality.

The present results showed that no improvement in initial baking quality resulted from cooling the product before packaging. Heat treatment studies have shown that rapid cooling of the product on removal from the drier is an essential (2).

Although the average value obtained for the baking volume of cakes from shell eggs of varying quality was 286 ml. (range 266 to 305 ml.), powder prepared on the laboratory drier produced cakes with baking volumes as high as 300 ml. This was attributed in part to the use of fresh shell eggs in this drier, but the major factor believed responsible was the use of an extremely small nozzle (0.025 in.).

While this study showed little difference in the initial quality of products prepared using solid sugar or sucrose syrup and using fresh eggs and frozen melange, it has been observed elsewhere that the use of syrup and the use of frozen melange resulted in products that were less stable when stored (2). In addition, the use of syrup necessitates the removal of a greater quantity of water, thereby increasing the cost of production.

Acknowledgments

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DRIED WHOLE EGG POWDER

XXIII. THE EFFECT OF MOISTURE CONTENT AND METHOD OF PACKING ON THE STORAGE LIFE OF DRIED SUGAR-EGG MIXTURES¹

BY R. L. HAY² AND JESSE A. PEARCE²

Abstract

Dried sugar-egg powders, obtained from a commercial Canadian source, were adjusted to 1.4, 2.8, and 3.2% moisture and stored at 40°, 80°, and 120° F from 1 to 52 weeks. Quality of the powder was assessed by measurement of fluorescence, potassium chloride value, pH, and foaming volume. The rate of deterioration increased with an increase in moisture content at 80° and 120° F. The effect of moisture content on fluorescence and potassium chloride values was negligible at 40° F, but high moisture in powders stored at this temperature accelerated the development of acidity and the loss in baking quality as assessed by foaming volume.

Packing in carbon dioxide, nitrogen, and *in vacuo* had a slight beneficial effect on dried sugar-egg powder.

Introduction

Lowering the moisture and volatile content of plain egg powder to 2% has been found to exert a definite beneficial effect on storage life (10). Moisture levels of less than 1% improved the keeping qualities of dried albumen and whole egg but not of dried yolk (7). In a recent investigation, sugar-egg powder tempered to a 1.4% moisture level was considerably better than a similar powder with a 2.8% moisture content, when held at elevated temperatures (1). Packing in carbon dioxide had a definite preservative action on stored plain egg powder but packing in nitrogen and under vacuum had no beneficial effect (9, 11).

The present paper deals with the effects of low moisture content and of packing with carbon dioxide, nitrogen, and *in vacuo* (inert packs) on the keeping quality of sugar-egg powder stored for one year.

Materials and Methods

The sugar-egg powder (33% sugar, dry basis) used in this investigation was similar to that described in an earlier paper (1) and was adjusted to moisture levels of 1.4, 2.8, and 3.2%. Samples at all moisture levels were sealed in an atmosphere of air; samples at 2.8% moisture were also sealed in carbon dioxide, nitrogen, and under vacuum. All were examined after storage for one, two, and four weeks at 120° F, after 1, 2, 4, 8, 16, and 32 weeks at 80° F; and after 16, 32, and 52 weeks at 40° F. The quality of the egg powder was assessed by measurement of fluorescence (4), potassium chloride value (8), pH (8), and foaming volume (1, 5).

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Contribution from the Division of Applied Biology, National Research Laboratories, Ottawa. Issued as Paper No. 169 of the Canadian Committee on Food Preservation and as N R C No. 1455

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During this investigation, it was observed that the foaming volume test was not entirely satisfactory and, as a result, a baking test has been substituted in other work in these laboratories (2). While foaming volume has proved inferior to the baking test, the former can be utilized to evaluate the baking quality of sugar-egg powder, when comparing powders from the same source.

Results

The results are presented in Figs. 1 to 4. It should be noted that the time intervals are shown as a geometrical progression, to permit graphical comparison of changes at high and low storage temperatures.

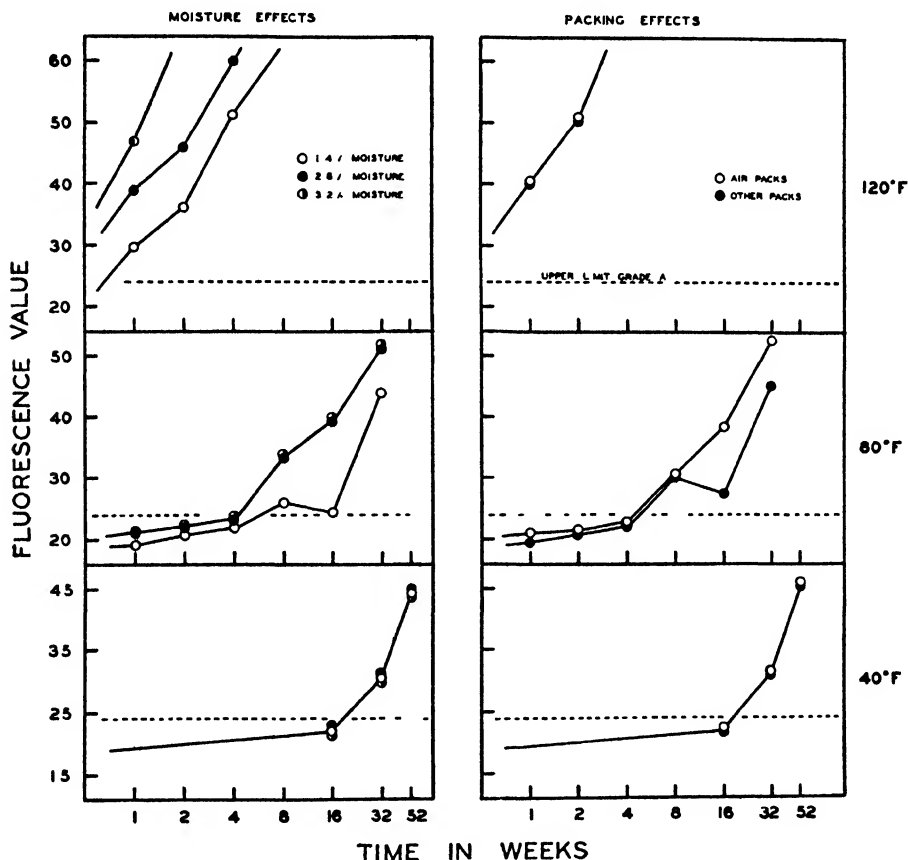


FIG 1 Effect of moisture contents of 1.4 to 3.2% and method of packing on the fluorescence values of dried sugar-egg powders

The Effect of Moisture Content

An increase in moisture content in the powder resulted in accelerated fluorescence development at 80° and 120° F. but had no measurable effect on the fluorescence of powders stored at 40° F. (Fig. 1). At 40° F. fluorescence

changes in the powders at all three moisture levels were negligible during the first 16 weeks, but fluorescence increased considerably and at equal rates for all moisture levels during the subsequent portion of the storage period. At 80° F. the fluorescence values of all powders increased slowly and at equal rates during the first four weeks, but subsequent changes in the 2.8 and 3.2% powders were much more rapid than in the 1.4% egg powder. At this temperature, reducing the moisture content from 2.8 to 1.4% appeared to

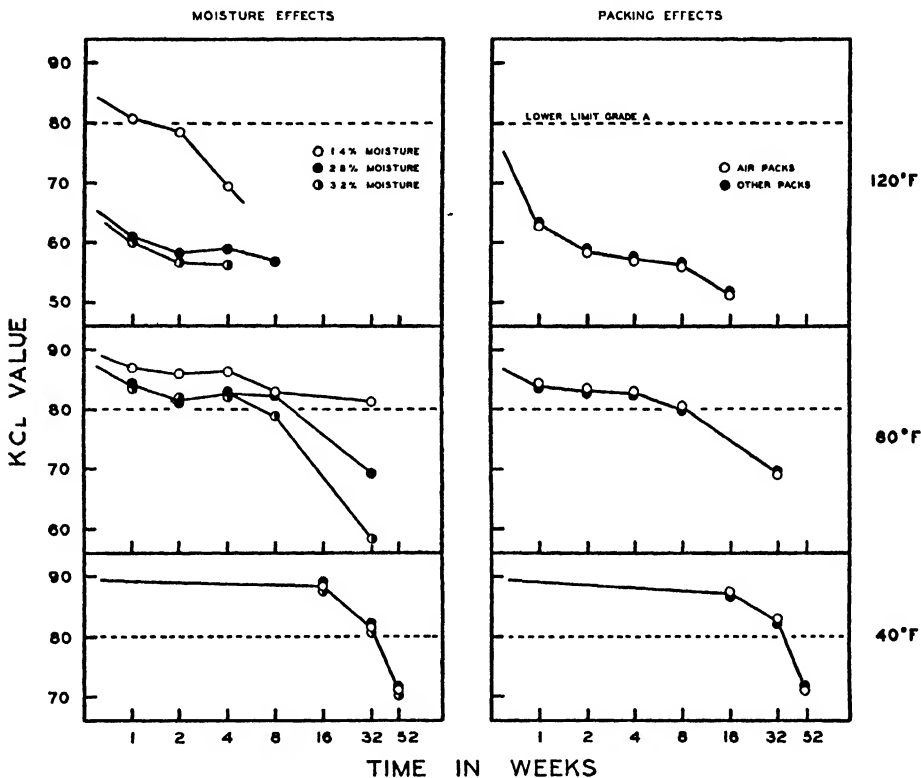


FIG. 2. Effect of moisture contents of 1.4 to 3.2% and method of packing on the potassium chloride values of dried sugar egg powders.

increase the storage life of sugar-egg powder by about 12 weeks. The difference in behaviour between the several moisture levels was most marked at 120° F., the 1.4% powder remaining at the lowest fluorescence level during the entire storage period.

The behaviour of the potassium chloride values in this study (Fig. 2) agreed with and supported the results noted above for the fluorescence test. Lowering the moisture content from 3.2 to 1.4% did not appear to prolong the storage life of sugar-egg powder when stored at 40° F. for one year. However, at both 80° and 120° F. the beneficial effects of reduction in moisture content from 3.2 to 1.4% were quite marked. At 80° F. loss in quality of the 1.4%

powder was comparatively small. The 3.2% samples deteriorated at approximately the same rate as those containing 2.8% moisture during the first four weeks of storage at 80° F., but showed a more rapid loss in solubility during the remaining portion of the storage period. Changes in potassium chloride value were rapid in all powders stored at 120° F., the most marked occurring in the 2.8 and 3.2% powders during the first week.

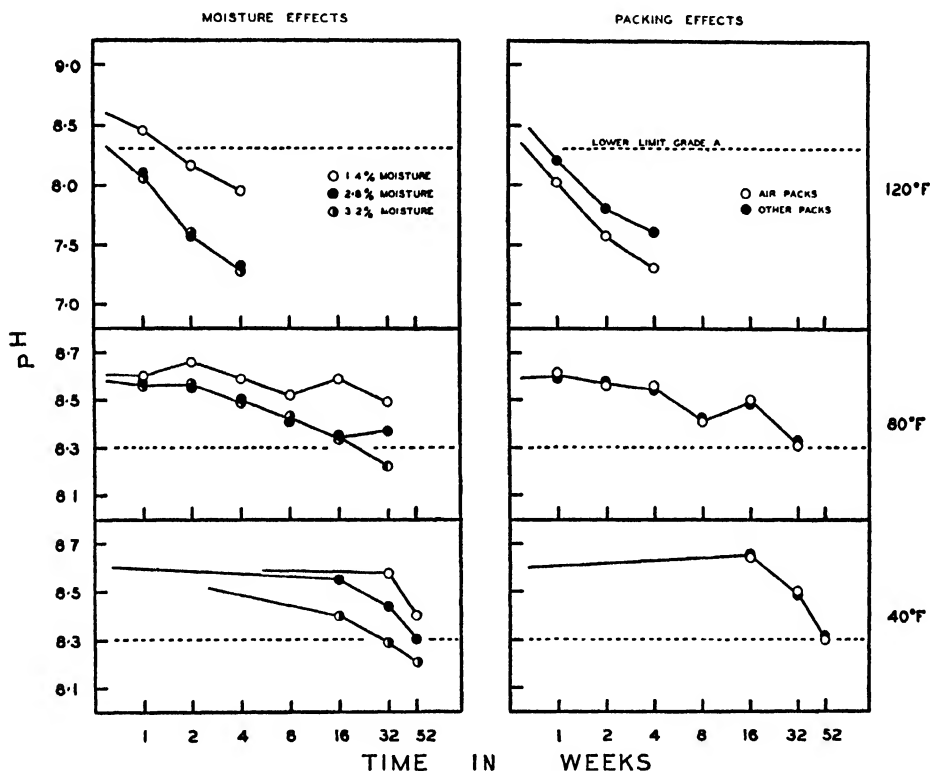


FIG. 3. Effect of moisture contents of 1.4 to 3.2% and method of packing on the pH values of dried sugar egg powders.

The pH measurements (Fig. 3) gave further evidence that very low moisture contents are advantageous. At all temperatures studied, powders containing 1.4% moisture maintained a higher pH level than either the 2.8 or 3.2% powders. There was little difference between the 2.8 and 3.2% powders at the higher temperatures, but, unlike the other tests, this test showed that reduced moisture content prolonged storage life at 40° F.

Notwithstanding the irregularities shown in Fig. 4, the foaming volume measurements supported the desirability of maintaining a low moisture content in stored sugar-egg powders. During a previous study (1), it was noted that sugar-egg powder with a high moisture content had a higher foaming volume after short storage periods than low moisture powder. However,

after the powders had been stored for some time the foaming volume of the high moisture powder decreased below that of the low moisture powder. In the present study (Fig. 4) a similar effect was evident during storage at 40° F.

Effect of Methods of Packing

Of the powders stored at 40° F. for 52 weeks there was no evidence by any test to show that gas-packing had a preservative effect on the quality of

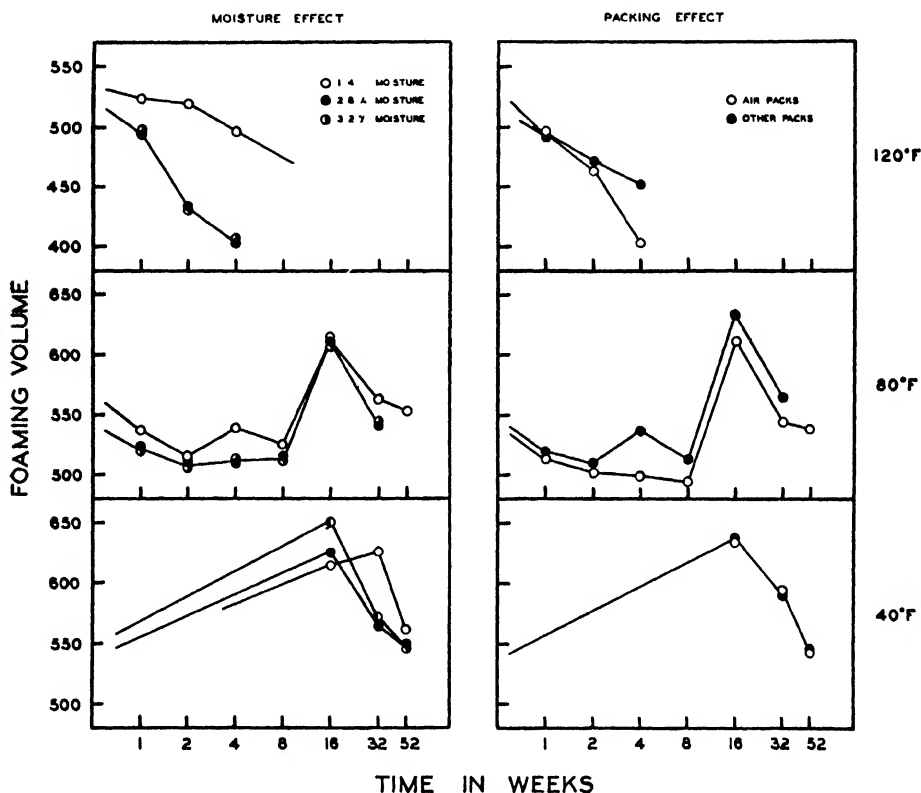


FIG 4 Effect of moisture contents of 1.4 to 3.2% and method of packing on the foaming volumes of dried sugar-egg powders

sugar-egg. At 80° F the fluorescence test (Fig 1) and the foaming volume test (Fig. 4) showed that the inert packs exerted some protective action during storage. However, pH (Fig. 3) and potassium chloride values (Fig. 2) were not affected by inert packing. Only pH and foaming volume measurements showed a beneficial effect from the use of inert packs on powders stored at 120° F. Although inert packs appeared to retard quality deterioration slightly, there was no evidence to show that any one was more effective than the other two. The limited protection provided by this treatment does not seem to warrant its use in current commercial practice.

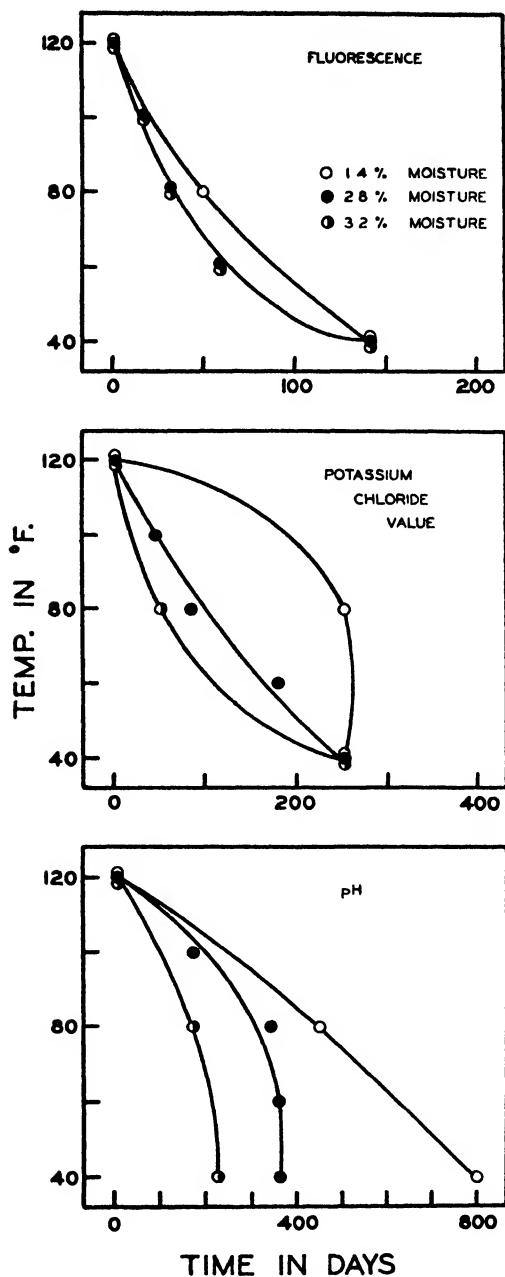


FIG 5 Effect of moisture contents of 1.4 to 3.2% on the time required for sugar-egg powder to change from A to B quality. Values plotted at 40°, 80°, and 120° F were calculated from data in Figs. 1, 2, and 3, those at 60° F were from the present study but not shown otherwise, those at 100° F from a previous study (1).

Discussion

The importance of moisture content in these powders can be assessed by considering the length of time required for them to change from *A* quality to *B* quality (shown by dotted lines in Figs. 1, 2 and 3 and summarized in Fig. 5) according to the tentative specifications for sugar-egg powder produced in Canada (6). Reducing the moisture content from 2.8 to 1.4% appeared to have little effect on the fluorescence and potassium chloride values of powder stored at 40° F.; perhaps, at this temperature, the protective effect of added sugar (1) was sufficient to mask any advantage gained from a very low moisture content. However, the pH changes indicated that the life of Grade *A* egg powder stored at 40° F. might be prolonged for more than one year when the moisture was reduced from 2.8 to 1.4%. Unless sugar-egg powder can be kept at temperatures of about 40° F. it seems advisable from these results to prepare powders with moisture contents below 2.8%, which are believed to be commercially feasible (2).

A previous investigation with plain egg powder showed that only carbon dioxide had a beneficial effect (11). The presence of added sugar in the egg powder used in this study apparently retards the reaction that contributes most to egg powder deterioration (probably a sugar-protein reaction (3)) and permits oxidation reactions to become more important. Hence any protective effect was common to all methods of obtaining an inert pack.

Acknowledgment

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LIQUID AND FROZEN EGG

III. SOME FACTORS AFFECTING THE QUALITY OF STORED FROZEN EGGS¹

BY JESSE A. PEARCE² AND MARGARET REID²

Abstract

Liquid from eggs of various qualities packaged in Reynold's Metal A-10 and liquid from Grade A eggs in wax paper with and without added ice was frozen at -40° F. and stored at 10° , 0° , and -10° F. for 12 months. Examination of baking properties and changes in pH, fluorescence, and reducing sugar content indicated the desirability of using liquid from Grade A eggs, although liquid from Grade C and cracked eggs may also be satisfactory; and of limiting the storage period for frozen egg, stored at these temperatures, to about six months. It was also desirable to allow the frozen egg to age for a month or two before use; and to use a highly moisture resistant barrier at all storage temperatures, although the wax paper and ice combination may be satisfactory at 0° and -10° F. Reducing sugar content decreased with an increase in the number of bacteria and, in addition, this measurement appeared to be a good indication of the quality of liquid and frozen egg.

Introduction

Since the production of eggs is seasonal, it is frequently necessary to carry large stocks for six months of the year or longer. The perishable nature of this commodity demands attention to the manner in which it is stored. While eggs in the shell can be held for short periods, some other method of preservation is desirable. Preserving eggs by removing them from the shell, mixing the yolk and white, and freezing has been an important commercial process for many years. During the war years, a large proportion of Canada's eggs were exported in the dried form; nevertheless, about five million pounds was frozen for use by bakery and other trades, exclusive of the quantities frozen for subsequent drying. However, only limited information is available to describe the keeping quality of the frozen product and the chemical changes occurring during its storage.

It seemed advisable before beginning the studies described in this paper to consider some of the changes likely to occur in eggs. An examination of frozen egg stored for six years at a temperature of 0° to -5° F. showed that if eggs of good quality were frozen, the odour of the product did not change but, if poor quality eggs were frozen, the initial putrid odour seemed to intensify (12). Although there appeared to be an increase in ammoniacal nitrogen as eggs became inedible (15, pp. 223-234), it seemed unlikely that this test would prove useful (15, pp. 260-261) and this was substantiated by preliminary work in these laboratories. As eggs deteriorate there is an increase in formic, lactic, and acetic acid content (5); therefore, measure-

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ments of pH changes seemed desirable. During work on dried eggs, it was observed that the fluorescence of extracts of the powder was related to the quality (9). Further work has indicated that fluorescence development may be attributed, in part, to a reaction between reducing sugars and proteins (1, 6, 7). Therefore, two modifications of this fluorescence test were applied to liquid eggs (10). It also appeared desirable to measure changes in reducing sugar content of the eggs, since this component has an important effect on the keeping quality of dried eggs (13) and since reducing sugars might disappear if the foregoing reaction occurred. In addition, sugar might also be removed by microbial growth (15, pp. 233-234). Much of the commercial frozen egg is used by the baking trade, and, for this reason, sponge cakes were believed desirable as tests of baking quality.

Materials and Methods

The storage experiment utilized material described in an earlier paper (10, Table IV). In brief, liquid from fresh Grade A eggs, from Grade A eggs held for 16 hr. at 80° F. in sterile glass containers, from Grade C* eggs, from cracked eggs, from musty eggs, and from "eight-day" incubator reject eggs (for grade descriptions, see (2)) was poured into moulds containing about four litres, frozen within 16 hr. in a room operating at -40° F. and held at temperatures of 10° (+ 2°, - ½°), 0° (± 1°), and - 10° (± 1°) F. for a period of one year. Samples were examined before and after freezing, and after 3, 6, and 12 months' storage. The liquid from fresh Grade A eggs was packed in Reynold's Metal A-10, a highly moisture vapour resistant material (16), plus a Fourdrinier kraft, B-Flute carton; in waxpaper (40 lb. kraft, waxed to 50 lb.) plus the carton with ice cubes (about 2 cu. in. in volume) inside the carton around the wrapped egg; and in wax-paper and carton without added ice. All other samples were packed in Reynold's Metal A-10 and cartons.

The analyses included measurements of pH and reducing sugar (4, pp. 416 and 438) on whole egg liquid; fluorescence of whole egg liquid, using a modification of a technique applied to egg powder (3); and baking volume and foaming volume measurements on whole egg liquid (11). In one portion of the study, reducing sugar content and pH of liquid egg, before and after freezing, were compared with the viable bacterial count (14).

In the initial stages of the study, pH, reducing sugar and fluorescence measurements were made on sera removed from the frozen egg by the chloroform treatment described earlier (10). It was possible to collect enough sera for all measurements on samples up to and including the three-month storage period. At the six-month storage period, only enough serum was separated from any one sample to permit fluorescence evaluation. The fluorescence changes up to the six-month sampling have been discussed (10) and at the 12-month storage period, the structure of the frozen egg had so changed that no sera could be separated.

* Eggs may be graded as C because of dirty shells or because of poor quality before the candling lamp (2); those used in this study were selected for poor quality.

Results

Effect of Bacterial Growth Before Storage on Reducing Sugar Content and pH

The relations between bacterial growth, reducing sugar content, and pH changes in frozen and unfrozen liquid egg are shown in Table I. Bacterial growth was most rapid in egg yolk and least rapid in egg white but both whole egg and egg yolk attained about the same bacterial populations after holding for 48 hr. at 80° F. Slight bacterial growth in liquid from whole egg and egg

TABLE I

THE RELATIONS BETWEEN REDUCING SUGAR CONTENT, pH, AND BACTERIAL GROWTH IN FROZEN AND UNFROZEN EGG

Material and treatment	Viable bacteria at 37° C.		Reducing sugar, %		pH	
	B.F.*	A.F.*	B.F.	A.F.	B.F.	A.F.
Whole egg						
48 hr. at 30° F.	<1 0 × 10 ³	1 0 × 10 ³	0 32	0 28	7 7	8 1
24 hr. at 80° F. and						
24 hr. at 30° F.	4 2 × 10 ⁴	2 2 × 10 ⁴	0 29	0 26	7 7	8.0
48 hr. at 80° F.	1 1 × 10 ⁵	1 8 × 10 ⁵	0 05	0 05	5 9	6 0
Egg white						
48 hr. at 30° F.	5 3 × 10 ³	2 0 × 10 ³	0 32	0 33	8 9	8 9
24 hr. at 80° F. and						
24 hr. at 30° F.	4 6 × 10 ³	7 7 × 10 ³	0 32	0 32	8 5	8 3
48 hr. at 80° F.	7 0 × 10 ⁴	2 2 × 10 ⁵	0 29	0 25	8 2	8 3
Egg yolk						
48 hr. at 30° F.	5 0 × 10 ³	5 0 × 10 ³	0 24	0 26	6 4	6.4
24 hr. at 80° F. and						
24 hr. at 30° F.	1 4 × 10 ⁴	1 4 × 10 ⁴	0 24	0 27	6 4	5 9
48 hr. at 80° F.	5 9 × 10 ⁵	3 0 × 10 ⁵	0 04	0 06	5 0	4 9

*B.F.—Before freezing. A.F.—After freezing.

white had a more marked effect on the sugar content than on pH, while, for separated yolk, fairly excessive bacterial growth had little effect on either measure. Freezing appeared to effect slight reduction in the bacterial content of some samples, and to have little effect on pH or reducing sugar content of liquid from egg white, but caused an apparent reduction in the reducing sugar content of liquid from whole egg and an apparent increase in the reducing sugar content of liquid from yolks.

Quality Changes During Storage

Baking volume, foaming volume, pH, and fluorescence measurements on the whole egg liquid were of little value in differentiating between many of the stored samples and were of less value in differentiating between storage temperature and between method of packaging. Baking or foaming volume measurements were of value in demonstrating the effect of storage time.

Volume measurements on sponge cakes made from the various types of liquid egg showed that musty eggs and incubator rejects were less suitable

than the other types of egg (Fig. 1). Liquid egg before freezing or frozen egg stored for three months gave larger cakes than egg just after freezing. At the six-month sampling, the baking volume was slightly less than that at the three-month sampling and at the twelve-month sampling it was markedly

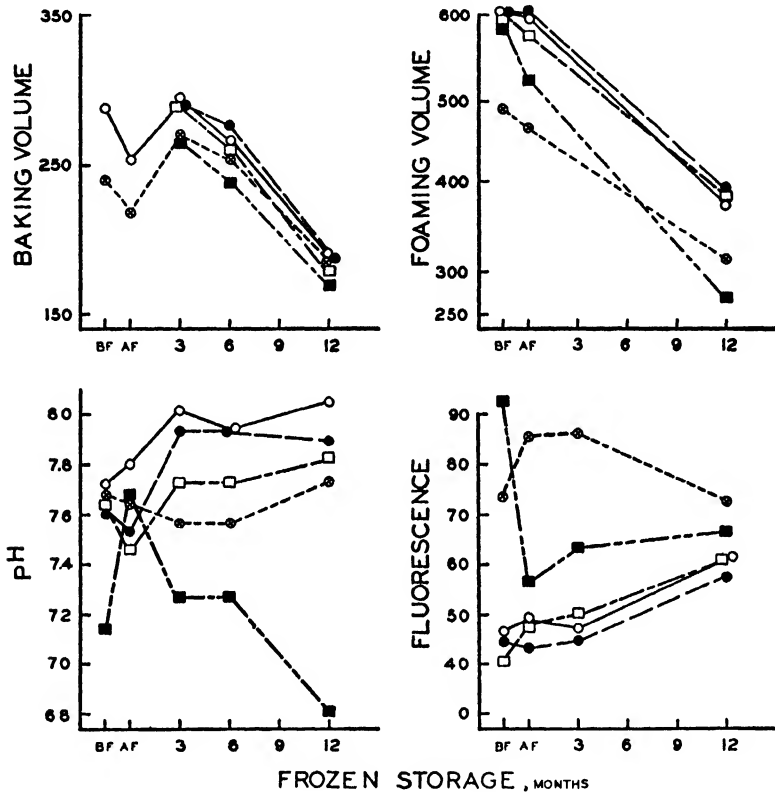


FIG. 1. Effect of freezing and frozen storage on baking volume (ml.), foaming volume (ml.), pH, and fluorescence of liquid from eggs of varying quality. ○ Grade A. ● Grade C. □ Cracks. ■ Musties. ⊕ Incubator rejects.

less. This reduction in the baking volume of egg when freshly frozen is known to occur in commercial practice and, in this state, the product is known as "green egg." Many concerns handling frozen egg prefer to let it age for a short period before releasing it.

Foaming volume measurements also showed that musty eggs and incubator rejects were less likely to be satisfactory in baked goods (Fig. 1). After 12 months' storage, egg held at 10° F. averaged 25 ml. less than the samples held at 0 or -10° F. Egg wrapped in Reynold's Metal A-10 and stored at 10 and 0° F. had foaming volumes 30 ml. greater than when wrapped in waxed paper (with or without added ice): at -10° F. no difference was evident.

Musty eggs were generally more acidic than incubator rejects, which in turn were more acidic than all other types of egg studied (Fig. 1). All types

of frozen egg, except that prepared from musty eggs, tended to become more alkaline as the storage time increased. Liquid from musty eggs increased in pH markedly during the freezing period and then decreased rapidly as storage progressed. Liquid from Grade *A* eggs suffered smaller pH increases when stored at -10° F. than when stored at 0° and 10° F. (Table II).

TABLE II

SOME EFFECTS OF TEMPERATURE ON THE QUALITY OF FROZEN EGG

Criteria	Type of eggs	Temperature, °F.	Storage time				
			B.F.*	A.F.*	3 Mos.	6 Mos.	12 Mos.
pH	Grade <i>A</i>	10	7 72	7 80	8 02	8 03	8.11
		0			8 08	7 87	8.08
		-10			7 92	7.90	7.95
Fluorescence	Incubator rejects	10	73 5	85 4	83 0	—	60 1
		0			88 0	—	76 1
		-10			86 0	—	80 9
	Musty	10	92 8	56 6	64 0	—	75 9
		0			62 0	—	62 0
		-10			64 0	—	61.4

*B.F.—Before freezing. A.F.—After freezing.

The fluorescence of material from Grade *A*, Grade *C*, and cracked eggs was generally lower than that of musty eggs and incubator rejects and showed a general increase through freezing and frozen storage, with the fluorescence value of Grade *A* and cracked eggs somewhat higher than the values for Grade *C* eggs (Fig. 1). The high fluorescence values of liquid from Grade *A* and cracked eggs when compared to those of liquid from Grade *C* eggs may reflect differences between measurements directly on the melange and measurements on the serum (10), or may be only a reflection of the limited number of samples of eggs used. In an earlier study, more comprehensive on this point, liquid from Grade *C* eggs, on the average, resulted in powders with fluorescence values higher than those of liquid from Grades *A* and *B* eggs (8).

Incubator rejects gave a product that increased in fluorescence during freezing and decreased in this attribute during frozen storage, while musty eggs gave a product that decreased in fluorescence during freezing and increased during frozen storage (Table II). The decreases for reject egg and the increases for musty egg were more rapid at $+10^{\circ}$ F. than at 0° and -10° F. While the fluorescence changes in the liquid from incubator rejects are at present unexplainable, the fluorescence and pH changes in musty egg may be related to commercial observations. If pails of liquid have a musty odour and are allowed to stand for about 24 hr. at 30° F., the musty odour will disappear. It is possible that the volatile products are acid in nature and

highly fluorescent. However, loss of these volatiles did not improve baking quality. An attempt is being made, in these laboratories, to isolate the possible acid, fluorescing volatiles.

Measurements of the reducing sugar content appeared to be of greater value than the foregoing and are shown in more detail (Fig. 2). These measure-

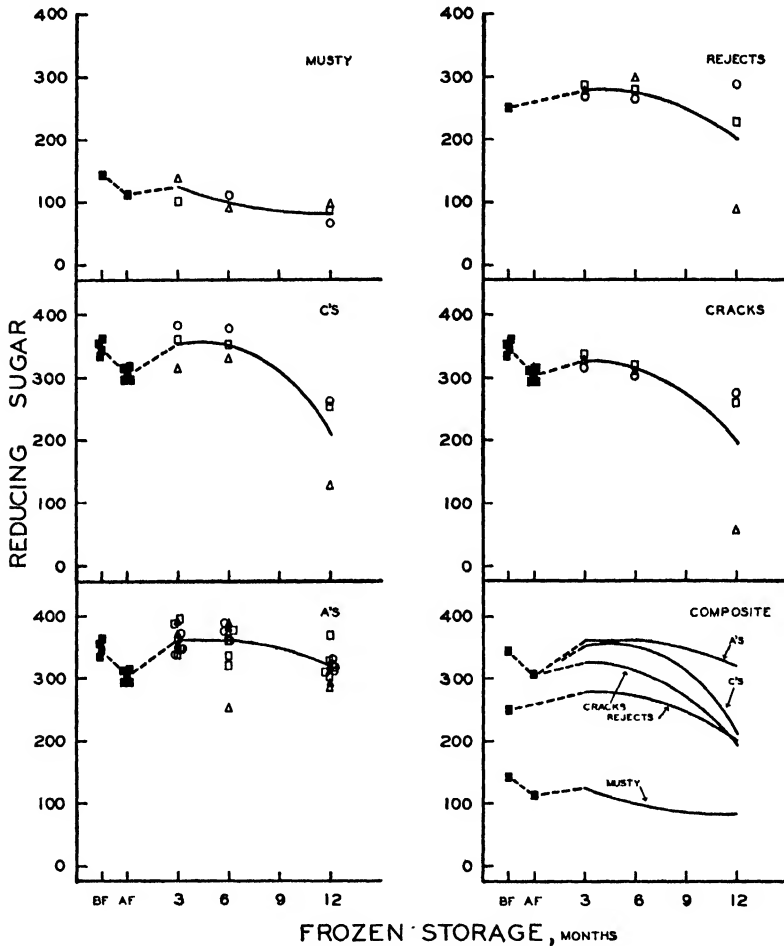


FIG. 2. Effect of freezing and frozen storage on reducing sugar content ($\% \times 1000$) of liquid from eggs of varying quality. ■ Values before (B.F.) and after freezing (A.F.). Δ Storage at 10° F. \square Storage at 0° F. \circ Storage at -10° F.

ments showed greater loss in sugar content as the storage temperature increased; as shown by the points in the figure, however, only mean changes for the various types of egg are given by the curves. The apparent decrease in reducing sugar content after freezing, observed in the preliminary study, was again noted here. This was followed by an increase after three months' storage but after six months' storage reducing sugar again decreased. These changes corresponded in general with the changes in baking volume.

The reducing sugar content was greatest for liquid from Grade A eggs and decreased for liquid from the other types in the following order: Grade C, cracked, incubator reject, and musty eggs. The reducing sugar values reported here for fresh liquid egg are in general agreement with some values reported in the literature, but lower than others (15, pp. 230 and 249). Measurements of reducing sugar appear to be a good indication of egg quality and they can be readily performed in a plant laboratory or by local consulting laboratories.

TABLE III

EFFECT OF PACKAGING METHOD ON FREEZER BURN IN FROZEN EGG STORED 12 MONTHS

Packaging method*	Approximate depth of dehydrated surface, in., at various temperatures		
	10° F.	0° F.	-10° F.
All samples in Reynold's Metal A-10	0	0	0
Liquid from Grade A eggs in wax paper plus ice chunks	>1	0	0
Liquid from Grade A eggs in wax paper only	$\frac{1}{2}$	$\frac{1}{2}$	$\frac{1}{2}$

* All further enclosed in kraft carton.

The effect of freezer burn on the frozen blocks is described in Table III. The greatest general protection was afforded by the Reynold's Metal wrap. The addition of chunks of ice reduced freezer burn at storage temperatures of 0° and -10° F. but appeared to accelerate freezer burn at +10° F. The latter effect may be attributable to differences in specific heat of the ice and the liquid egg, resulting in transfer of moisture from the blocks of frozen egg to the ice as the temperature of the storage room and its contents varied around the controlled temperature, 10° (+2°, - $\frac{1}{2}$ °) F.

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DRIED MILK POWDER

VI. THE EFFECT OF GAS- AND VACUUM-PACKING ON KEEPING QUALITY¹

BY JESSE A. PEARCE² AND W. A. BRYCE³

Abstract

Skim (1% fat) and whole (26, 28, and 30% fat) milk powders (2% moisture) from two plants were packed in air, carbon dioxide, nitrogen, 80% carbon dioxide and 20% nitrogen, 20% carbon dioxide and 80% nitrogen, and under vacuum, and stored for 12 months at 80° F. Quality was assessed by a tasting panel of 14 persons. Packing in an inert gas or under vacuum effected a general improvement in the quality of skim-milk powders. This was attributed to removal of volatile degradation products during the packing process and early storage. The storage life of whole milk powders was increased from a maximum of three months when packed in air to nine months when packed in inert gases or vacuum.

Introduction

Packing milk powders in nitrogen or carbon dioxide or in mixtures of both has become common commercial practice, but the effectiveness of these gases for preserving milk powders has been the subject of some controversy. The use of nitrogen only was reported to have no beneficial effect (14), but other investigations indicated that it provided protection (15), particularly if the oxygen concentration of the headspace gas was maintained at a low level (5, 8). The use of carbon dioxide has been variously reported as favourable (8, 15), without beneficial effect (14, 16), and harmful (7) to stored, dried, whole milk. Other reports indicated that mixtures of these two gases provided protection to stored milk powders (3, 6) and that packing under vacuum or partial vacuum had a beneficial effect on stored milk powders (7, 14, 15).

Studies on dehydrated egg-and-milk mixtures have shown that packing in carbon dioxide extends the storage life of this product (13). Carbon dioxide had a greater preservative effect on stored egg powder than nitrogen, which was in turn better than air (12).

The above results show that marked disagreements exist in the published investigations on the effect of gas-packing on the storage life of dried milk. It was, therefore, deemed advisable to compare the effect of packing in air, carbon dioxide, nitrogen, mixtures of carbon dioxide and nitrogen, and under vacuum, on milk powders of different fat levels from different sources. The present paper describes this investigation.

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Materials and Methods

The materials used were those described in an earlier paper of this series (1) and consisted of powders of 1, 26, and 28% butterfat from one plant and powders of 1, 26, 28, and 30% butterfat from another. The powders were tempered to a moisture content of 2% by vacuum desiccation over phosphorus pentoxide and were packed in tinplate containers.

Occluded air (oxygen) may effect the storage life of gas-packed milk powder (3, 6, 8, 9). Therefore, in the packing technique used, an attempt was made to reduce the occluded gas to a minimum and to have the same amount in each sample. Using a previously described apparatus (2), the chamber containing the tins was evacuated to 1 mm. pressure, flooded with gas of the desired composition, evacuated as before, flooded again, and sealed. In vacuum packing, the tins were held under 1 mm. pressure for 15 min. and sealed at this pressure. The gases used were nitrogen, carbon dioxide, 80% carbon dioxide and 20% nitrogen, and 20% carbon dioxide and 80% nitrogen. Although it is believed that carbon dioxide may not be an "inert" gas when used to protect stored fat (7), the gas and vacuum-packs will be grouped under the heading "inert packs."

The foregoing samples and control samples with air as the headspace gas were stored at 80° F. and examined by palatability assessment initially and after 1, 3, 6, 9, and 12 months. To assess palatability, the samples were reconstituted as previously described (10), and sampled by 14 tasters. Scoring was done on a scale of 10 (the equivalent of excellent, fresh whole or skim-milk) to 0 (repulsive). A score of 4 is considered the point at which milk is no longer suitable for use as a milk drink. The reliability of the scoring by the taste panel has been estimated and palatability assessment was found to be more suitable than any of the chemical tests of milk powder quality (10). The desirability of using organoleptic tests as well as chemical tests on stored milk powders has been observed by others (4).

Results

The scores for the skim-milk powders (1% butterfat) at the 1 to 12 month samplings and scores for the whole milk powders (26, 28, and 30% butterfat) at the 1 to 9 month samplings were subjected to analysis of variance. The factors found to be significant are shown, in Fig. 1, by curves drawn through the mean palatability values for the various sampling times.

As noted previously, these skim-milk powders improved in quality during the first month of storage (1). Contrary to these earlier results (1), skim-milk powders from the two sources, when stored in an atmosphere of air, did not differ significantly in quality, but after 12 months' storage in inert atmospheres or in vacuum the powder from Plant 2 was considered half a palatability unit better than powder from Plant 1. Although no one method of obtaining an inert pack was significantly better than any other, after 12 months' storage, skim-milk powders in inert packs were significantly better than the air-packed material (about one palatability unit).

The air-packed whole milk powders deteriorated in a manner similar to that previously described (1); powders of 26 and 28% fat from Plant 2 had the poorest keeping quality; powder of 30% fat was better; and powders of 26 and 28% fat from Plant 1 had the best storage life. As observed in the previous study (1), there was no significant difference between powders of 26 and 28% fat from either plant.

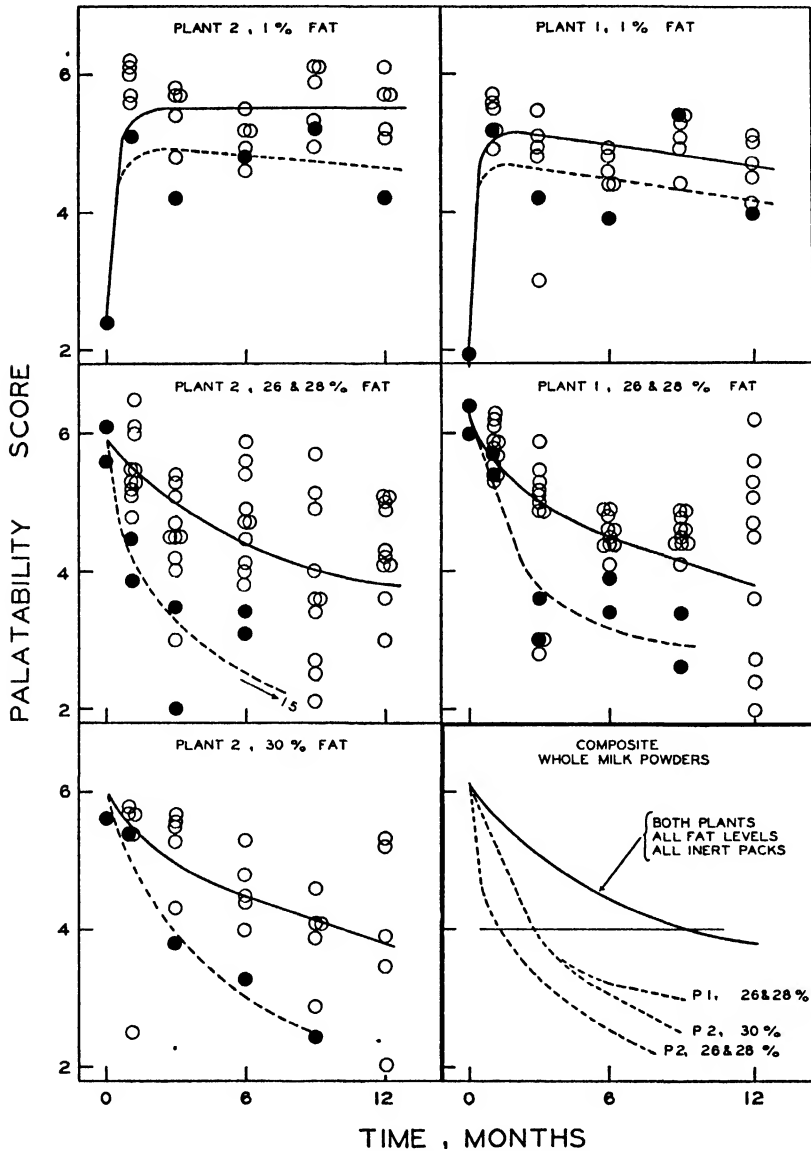


FIG. 1. The effect of gas- and vacuum-packing on skim and whole milk powders stored at 80° F.

The average loss in quality was the same in all whole milk powders packed in an inert atmosphere or under vacuum. No one method of obtaining an inert pack lengthened storage life significantly more than any other. However, the use of inert packs extended the average life of whole milk powders, normally one to three months, to nine months.

Discussion

The consistently higher palatability scores for the skim-milk powders packed in inert atmospheres and under vacuum support a previous suggestion that degradation products, responsible for low quality, are dissipated during the first few weeks after repacking (11). Beneficial effects of inert packing were evident after one month, and this superiority was maintained throughout the storage period. It is possible that subjecting these powders to low pressures during repacking removed quantities of volatile degradation products in excess of those dissipated when repacking in air only.

The variation in the rate of quality deterioration of the air-packed whole milk powders shows the effect on fat stability of processing practice in the different plants. Gas- or vacuum-packing reduced this difference in fat stability. While dissipation of degradation products may have been partly responsible for this improved storage life of gas- or vacuum-packed whole milk powders, the increasing differences in palatability, as storage progressed, supports previous evidence (5, 8) that reduction in the oxygen content of the gas surrounding the powder particles minimizes deterioration.

Deteriorative changes in whole milk powders packed in inert atmospheres approached those of air-packed skim-milk powder. This and other factors (11) indicated that solids-not-fat also play an important role in the deterioration of whole milk powders.

A wide variation was observed in the scores applied to the powders packed in inert gases or under vacuum. This variation includes the effect of a number of factors: source of powder, fat level, method of obtaining an inert pack, variability in the milk powder samples, and variability of taster scores. These results may explain the observed differences in effectiveness of methods of gas-packing. Examination of the scatter shows that some samples in inert packs were judged to be of lower quality than air-packed samples, while others were considered to be about the same quality as the air-packed samples. Therefore, it is possible that samples in inert packs, when examined by one investigator, might appear no better than air-packed samples, while another examination would show inert packing to have a beneficial effect. The results presented here show that the average effect of inert packing is a prolongation of the storage life of dried milk powder.

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A STUDY OF METHODS FOR ASSESSING RANCIDITY IN LARD¹

BY G. A. GRANT² AND H. J. LIPS^{3*}

Abstract

Lard from 26 sources was stored in glass jars at 26 7° C (80° F) until definitely rancid. Spoilage was evaluated at two-week intervals by chemical tests and odour ratings. Correlation coefficients between odour scores and the logarithms of chemical test values were: iodometric peroxide, - .90; alpha-dicarbonyl test, - .85; Stamma test - .82; Kreis test - .81; ferrometric peroxide, - .80; fluorescence, .79; free fatty acids, - .10. Association between chemical measurements was greatest between alpha-dicarbonyl and iodometric peroxide values ($r = .97$). As peroxides are not thermostable, the measurement of the stable alpha-dicarbonyl compounds, although less precise, is considered the best available chemical method for assessing rancidity.

Introduction

Recent increases in production and export of Canadian lard have focused attention on the perishability of the product. As part of a program to improve the stability and general quality of lard, a study was made of available chemical methods for detecting rancidity. A number of these methods and their association with odour ratings are described in the present paper.

Methods

The exact measurement of rancidity development in fats by any one procedure is difficult. This is due to the diversity of the reactions producing rancidity, e.g., atmospheric oxidation and the action of micro-organisms and enzymes. Taste and smell, among the least sensitive of the senses, have been widely used as criteria of rancidity but since odour and taste judgments are difficult to reproduce, it is desirable to employ chemical or physical measurements, which are reproducible and can be calibrated against the results of odour or taste panels.

Considerable uncertainty exists concerning the relative merits of the chemical tests and only those showing promising results from preliminary trials were selected for the present study. These included determination of peroxide oxygen, Kreis, Stamma, alpha-dicarbonyl, and fluorescence values.

Peroxide Oxygen Content

Several methods have been proposed for determining peroxides in fats (1, 5, 6, 11, 22). A modification (6) of an iodometric method (10) and a modification (11) of a ferrometric method (1) were selected. The iodometric is

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simpler than the ferrometric procedure but the latter has been reported to be more sensitive.

A previous study on chicken fat indicated that peroxide oxygen content as determined by the iodometric procedure was 20% less than the actual content (2) because iodine was absorbed by the unsaturated fatty acids. As lard has an appreciably lower iodine number than chicken fat, it was assumed that this correction was not necessary. Lea's original practice (10) of reporting peroxide oxygen as millilitres of 0.002 *N* sodium thiosulphate is employed in this investigation for the iodometric procedure. The peroxide content by the ferrometric method (11) is reported as milliequivalents of peroxide per kilogram of fat.

Kreis Value

Of the several modifications of the Kreis test (9) one (20) was selected because of its simplicity and because the colour was developed in a single phase system. The colour intensity has been found proportional to the concentration of fat (21), increasing with a decrease in fat concentration. For this investigation the same concentration of fat, 1 gm. in 10 ml., was adopted for all determinations, dilution, if necessary, being made in the coloured solution. The Evelyn photoelectric colorimeter (3) was used for all colorimetric procedures. The colour intensity of the Kreis test was determined using a No. 540 $m\mu$ Rubicon filter, and reported as extinction coefficients according to the equation $E = \frac{2 - \log G}{C}$, where G is the corrected galvanometer reading and C is the concentration of fat in grams per millilitre of final solution. Although this value has little physical significance it is suitable for purposes of comparison.

Alpha-dicarbonyl and Stamm Values

Many tests for rancidity depend on the presence of aldehydes and ketones in the oxidized fat. Of the many methods for the detection of aldehydes and ketones in rancid fat (4, 8, 12, 16, 17), two (12, 17) were chosen for further study.

The Stamm method (17) used arbitrary standards to measure the colour developed, and was not sensitive to small changes in colour intensity. Use of a colorimeter was believed desirable to obtain accurate comparison between samples. The reagent was prepared by heating 0.5 gm. of *s*-diphenyl-carbazide in 100 ml. of tetrachloroethane until it dissolved, cooling rapidly, and filtering in a darkened room. It was then stored in a brown reagent bottle. Heating 1.0 gm. of fat with 10 ml. of reagent in a graduate for 3.0 min. at 100° C., cooling rapidly, and reading immediately in the Evelyn colorimeter employing a 580 $m\mu$ filter was found to be satisfactory. The results are reported as extinction coefficients.

The alpha-dicarbonyl method (12) was also modified for use with the Evelyn colorimeter. Preliminary test solutions containing 1.0 gm. of fat, 1.0 ml. of 30% potassium hydroxide solution, and 9.0 ml. of ethanol heated for 20 min. at 60° C., demonstrated differences between fresh and rancid lard.

However, the solutions were not suitable for reading in the colorimeter, as they separated into two phases. Employing stronger potassium hydroxide solution or heating for one hour failed to produce a single phase system, but with the use of 30 ml. of alcohol the solutions remained clear and in a single phase.

To investigate the effect of fat concentration on the extinction coefficient, 0.5, 1.0, and 3.0 gm. of lard were heated with 3.0 ml. of potassium hydroxide solution and 32 ml. of purified ethanol. The solution containing 3.0 gm. of lard became cloudy and had to be filtered. The ethanol was freed of aldehydes and ketones by refluxing with calcium oxide, distilling, shaking with 2,4-dinitrophenylhydrazine and redistilling. The results are given in Table I. The extinction coefficient decreased with an increase in fat concentration and 1.0 gm. of fat showed the largest difference between fresh and rancid lard.

TABLE I
THE EFFECT OF FAT CONCENTRATION ON EXTINCTION COEFFICIENTS IN THE ALPHA-DICARBONYL TEST

Weight of sample, gm.	Extinction coefficients	
	Fresh	Rancid*
0.5	3.2	7.1
1.0	0.9	5.4
3.0	0.9	3.7

* The peroxide oxygen content of the rancid sample was 14 ml. of 0.002 N thiosulphate per gm.

The effects of temperature and time of heating were also investigated. Solutions were heated for 30 min. at 60° C. and 100° C. and for 30, 60, and 90 min. at 80° C. The results are shown in Tables II and III. Raising the

TABLE II
THE EFFECT OF TEMPERATURE AFTER HEATING FOR 30 MIN. ON THE ALPHA-DICARBONYL EXTINCTION COEFFICIENTS

Temperature, °C.	Extinction coefficients	
	Fresh	Rancid
60	0.2	9.4
80	0.7	8.4
100	0.4	9.1

temperature from 60° to 100° C. had little effect on the extinction coefficient, but increasing the time of heating resulted in a higher value. This effect was more pronounced in fresh lard than in the rancid samples.

The procedure adopted was as follows. One gram of fat was weighed into a glass-stoppered graduate and 3 ml. of 50% potassium hydroxide solution and 30 ml. of purified ethanol were added. The solution was heated for 30

TABLE III

EFFECT OF TIME OF HEATING AT 80°C ON ALPHA-DICARBONYL EXTINCTION COEFFICIENTS

Time, min	Extinction coefficients	
	Fresh	Rancid
30	0.72	8.4
60	1.70	8.9
90	2.12	9.2

min. at 80° C, then allowed to cool, made up to 35 ml. with alcohol, and read in an Evelyn colorimeter using a 420 m μ filter. The results were reported as extinction coefficients.

Fluorescence

Examinations of lard by ultra-violet light have been reported (13; 19, pp. 90-91). A method (13) using the Coleman photofluorometer standardized with quinine sulphate as previously described (14) was investigated. Fluorescence values are reported as photofluorometric readings of the lard solution minus blank solvent readings.

From previous work on butterfat (7) it was indicated that the type of solvent and concentration of fat might have a marked influence on the fluorescence values. To study the effect of organic solvents on fluorescence of fresh and rancid lard, 1.0 gm. of lard was dissolved in 10 ml. of various solvents, slightly warmed, mixed thoroughly, and read on the photofluorometer. Petroleum ether, xylene, dioxane, benzene, and ethylene dichloride were studied, as the previous work had indicated a better differentiation between rancid and fresh fat with these solvents. The results are given in Table IV. The rancid lard gave lower fluorescence values with all solvents.

TABLE IV

THE EFFECT OF ORGANIC SOLVENTS ON THE FLUORESCENCE OF FRESH AND RANCID LARD

Solvent	Fresh	Rancid
Petroleum ether	14.0	12.0
Xylene	19.5	12.0
Dioxane	16.5	8.7
Benzene	16.0	8.0
Ethylene dichloride	15.5	11.5

Greater differences were observed with dioxane, benzene, and xylene than with petroleum ether or ethylene dichloride. Xylene was chosen for further study as it gave the lowest blank reading.

To study the effects of concentration, 1- to 5-gm. samples of fresh or rancid lard were dissolved in 10 ml. of xylene. The results are given in Table V.

TABLE V
THE EFFECT OF CONCENTRATION ON THE FLUORESCENCE
OF FRESH AND RANCID LARD

Weight, in gm. per 10 ml. of solvent	Fluorescence value	
	Fresh	Rancid
1	13.7	8.7
2	27.5	12.2
3	37.2	15.0
5	Over 100	16.0

An increase in fat concentration gave an increase in fluorescence values. This increase was of greater magnitude with fresh lard than with the rancid samples. As the fluorescence changes between the fresh and rancid lard were not similar with dilution, an empirical method was adopted to ensure comparability. One gram of fat was weighed into a 10 ml. glass-stoppered graduate and 10 ml. of xylene added. The mixture was shaken until the fat was completely dissolved and then read in a Coleman photofluorometer.

Odour Tests

Rancidity in the lard was assessed by a 10 member panel and scored on the following basis: 10, excellent, odour fresh or absent; 8, good, no rancid odour; 6, fair, slight rancid odour; 4, poor, odour definitely rancid; 2, bad, odour very rancid; 0, unapproachable. Odours that could be classified as burnt, tanky, or otherwise objectionable, but not rancid, were given a rating of 7 on this scale.

Materials and Procedure

Samples of all types of lard manufactured in Canada were received from 26 Canadian packing plants and stored in half-pint glass jars at 26.7° C. Ten samples were put into storage every two weeks and sampled at two-week intervals. This ensured differences in level of rancidity, and convenience in the number of samples coming out of storage at any one time. Samples for odour tests were removed and the remainder melted on a steam-bath and mixed thoroughly to provide material for the objective tests. If all the tests were not completed on the day of sampling the material was stored at -40° C.

Results

The association between objective tests and the odour test was assessed by computing simple correlation coefficients. For predicting odour scores from objective test values, the correlation coefficient must be highly significant and attain a value of .8 to .9. A small scatter around the regression line is also desirable and this was assessed by computing the error of estimate. The coefficients of correlation with their errors of estimate and prediction equations between odour score and the logarithms of the objective tests are given in Table VI.

TABLE VI

THE CORRELATION COEFFICIENTS, PREDICTION EQUATIONS, AND ERRORS OF ESTIMATE BETWEEN ODOUR SCORE AND LOGARITHMS OF THE OBJECTIVE TEST DATA

Quantities correlated with odour score.	Degrees of freedom	Correlation coefficients	Prediction equations	Errors of estimate
Alpha-dicarbonyl	204	— .85	$y = 8.48 - 2.78x$	0.83
Iodometric peroxide oxygen	160	— .90	$y = 7.63 - 1.81x$	0.70
Ferrometric peroxide oxygen	204	— .80	$y = 8.30 - 1.52x$	0.94
Kreis	183	— .81	$y = 10.0 - 2.73x$	0.54
Stamm	204	— .82	$y = 7.67 - 2.01x$	0.91
Free fatty acids	108	— .10	—	—
Fluorescence	191	.79	$y = 5.53 + 6.63x$	0.96

A decrease in odour score was associated with an increase in all the objective values, except the fluorescence measurements, which gave a corresponding decrease. Of the objective tests correlated with rancidity as assessed by odour scores, the alpha-dicarbonyl values and peroxide oxygen determined by the iodometric procedure gave the highest associations. The Kreis, Stamm, ferrometric peroxide oxygen, and fluorescence values were all about equally associated with odour score. The sensitivity of the tests as assessed by the regression coefficients showed the ferrometric peroxide oxygen to be most sensitive. However, this test and the fluorescence measurement had the two largest errors of estimate. The fluorescence values, although highly associated with odour score, had regression values too high to enable prediction of odour scores lower than 5.0 (Fig. 6), the fluorescence values being almost nil at this point. This indicates that fluorescing substances were almost completely destroyed when the lard had just become rancid. The free fatty acid values were not significantly associated with odour scores.

Further details of the association between objective tests and odour scores are shown in Figs. 1 to 6. The equations for the relations shown in the figures are given in Table VI. It is evident from Figs. 1 to 6 and Table VI that a slightly rancid lard (odour score of 6) corresponded to the following objective test values: alpha-dicarbonyl value, 7.8; iodometric peroxide oxygen, 7.8; ferrometric peroxide oxygen, 31.6; Kreis value, 28.8; and Stamm value 6.8. The regression lines of the objective tests are shown in Fig. 6. The two

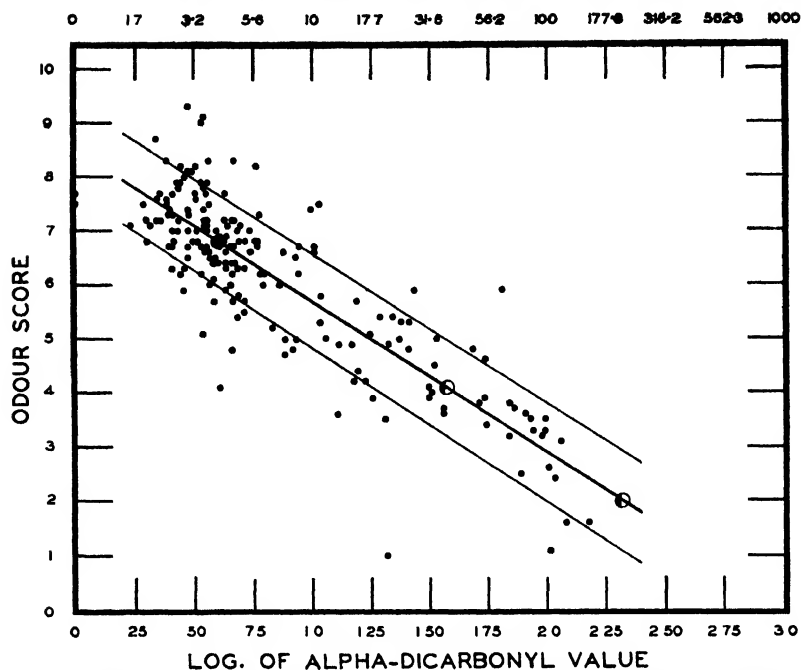


FIG 1 Relation between odour score and alpha dicarbonyl value on development of rancidity in lard stored at 80° F

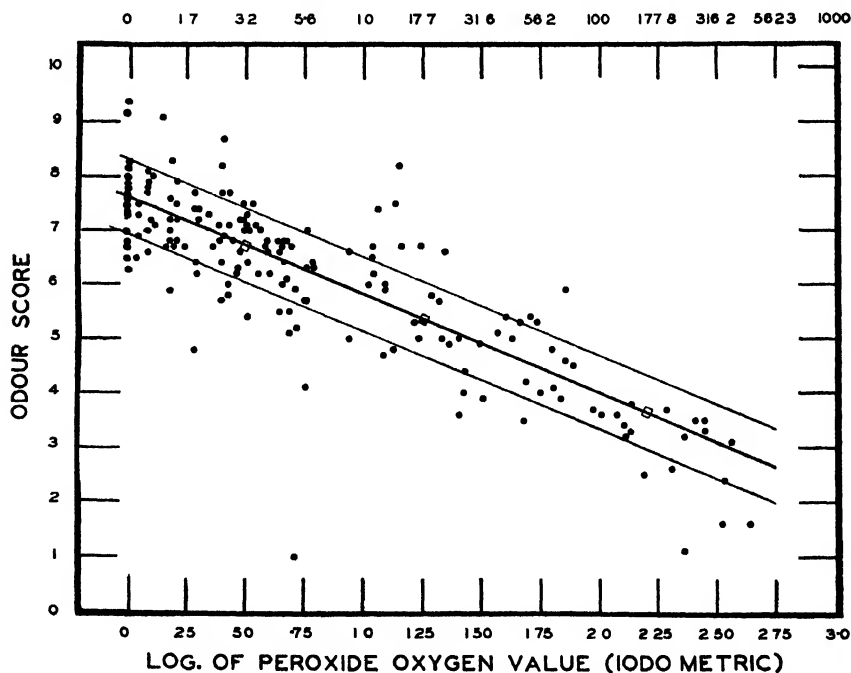


FIG 2 Relation between odour score and iodometric peroxide oxygen content on development of rancidity in lard stored at 80° F

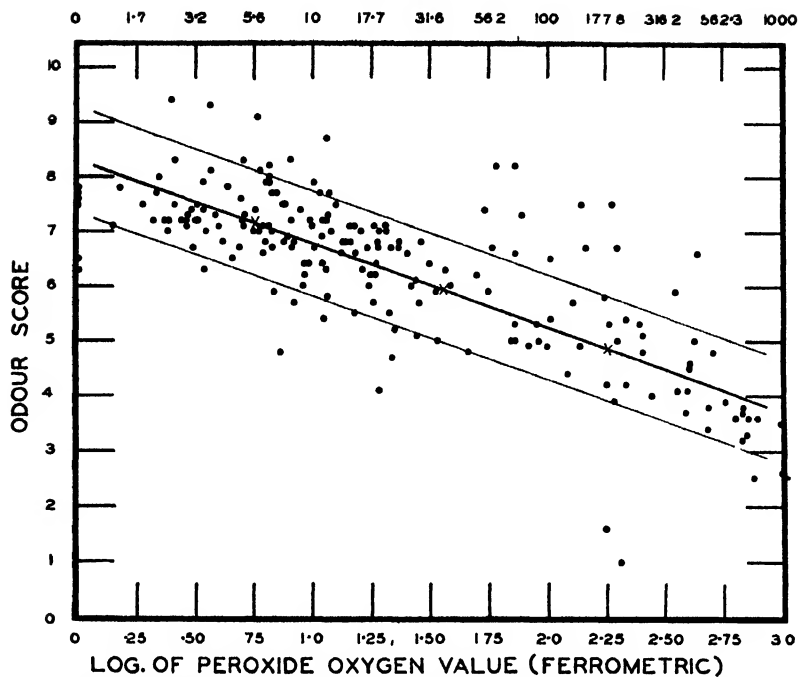


FIG. 3. Relation between odour score and ferrometric peroxide oxygen content on development of rancidity in lard stored at 80° F.

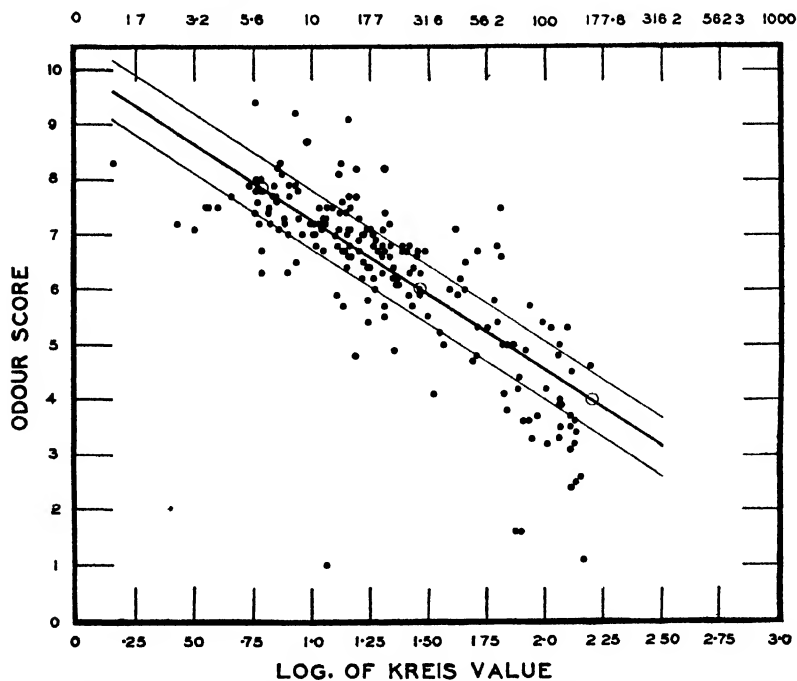


FIG. 4. Relation between odour score and Kreis value on development of rancidity in lard stored at 80° F.

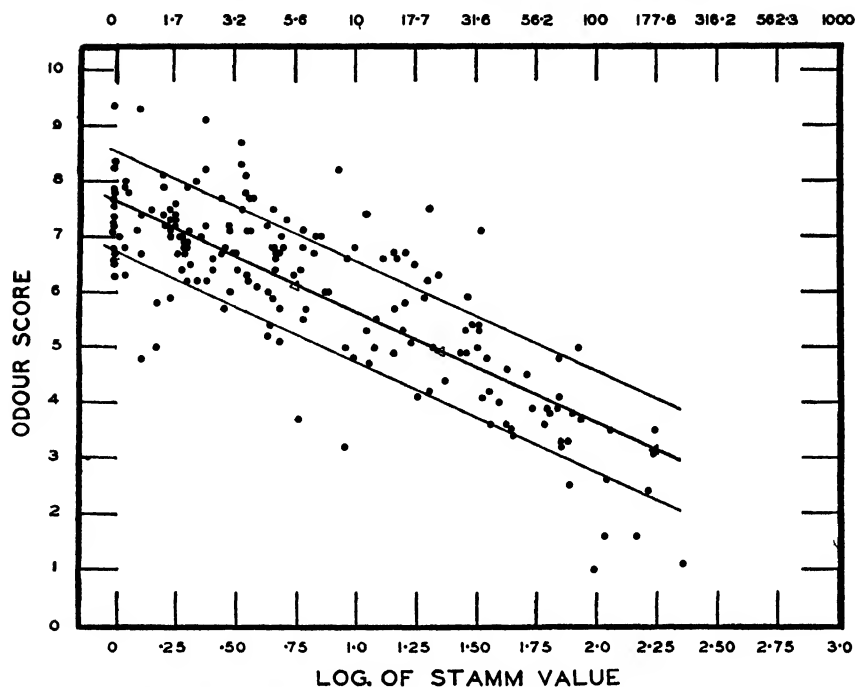


FIG. 5. Relation between Stammm value and odour score on development of rancidity in lard stored at 80° F.

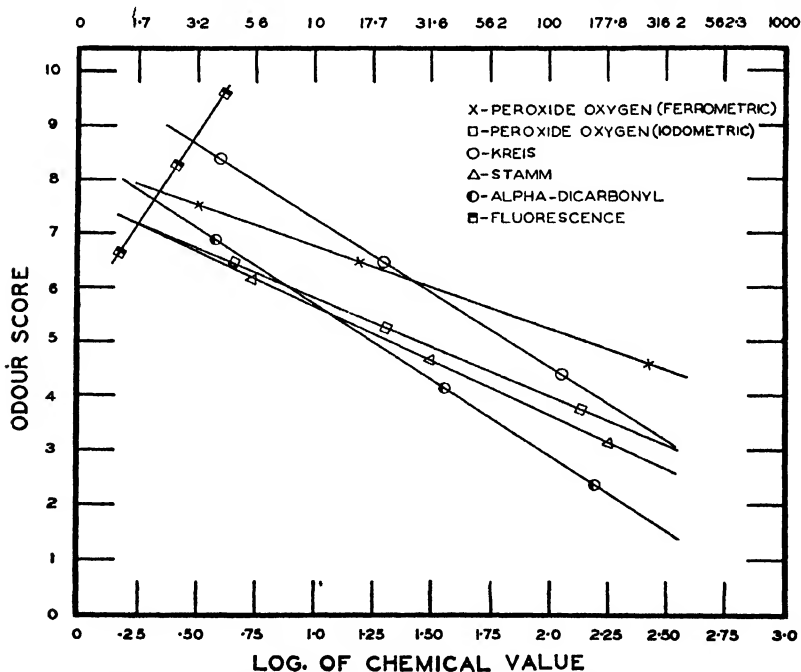


FIG. 6. Relation of regression lines of the objective tests and odour score in lard which developed rancidity on storage at 80° F.

peroxide methods gave the same slope. The Kreis and alpha-dicarbonyl measurements also showed equal slopes, but of greater magnitude than those of the peroxide values.

Interrelation of Chemical Measurements

The interrelation of the chemical measurements was assessed by computing simple correlation coefficients (Table VII). Of the methods investigated, the

TABLE VII

SIMPLE COEFFICIENTS OF CORRELATION BETWEEN OBJECTIVE TESTS ON LARD
THAT ATTAINED STATISTICAL SIGNIFICANCE

Quantities correlated	Degrees of freedom	Correlation coefficients
Alpha dicarbonyl value with		
Iodometric peroxide oxygen content	204	0.97**
Ferrometric peroxide oxygen content	204	0.86**
Stamm value	204	0.60**
Logarithm of alpha dicarbonyl value with		
Kreis value	198	0.89**
Iodometric peroxide oxygen content with		
Stamm value	204	0.62**
Ferrometric peroxide oxygen content	204	0.84**
Logarithm of iodometric peroxide oxygen with		
Logarithm Kreis value	198	0.90**
Ferrometric peroxide oxygen content with		
Stamm value	204	0.60**
Logarithm of ferrometric peroxide oxygen with		
Kreis value	204	0.57**
Stamm value with		
Kreis value	204	0.73**

** Indicates 1% level of statistical significance

alpha-dicarbonyl value and peroxide oxygen content were most closely associated. The Kreis values showed a logarithmic association with alpha-dicarbonyl and peroxide oxygen content. The results suggest that formation of peroxides is more closely associated with alpha-dicarbonyl compounds, believed present in increasing quantities in rancid fat (16), than with epiphydrin aldehyde, which may also be present (15), and which is supposedly responsible for the Kreis test.

Discussion

Although most of the results of the chemical methods were highly associated with those of organoleptic rancidity, the peroxide oxygen and alpha-dicarbonyl measurements appeared to have more advantages than the others. As peroxides are not thermostable, the peroxide oxygen content is usually altered by

processing techniques such as deodorizing or bleaching. Thus, substantial oxidation may have taken place, but the material may have only a small peroxide value. The measurement of the stable alpha-dicarbonyl compounds although less precise is considered a better method for the assessment of rancidity.

It is of interest to note the disappearance of fluorescent materials with the appearance of rancid odours at the end of the induction period. This indicates that fluorescence in lard may be linked with natural antioxidant substances, which are altered by oxidation.

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A FLUORESCENCE METHOD FOR ASSESSING THE KEEPING QUALITY OF BUTTER¹

BY G. A. GRANT² AND W. HAROLD WHITE³

Abstract

The fluorescence values of serum from salted butter were affected by separation temperature, dilution, nature and pH of the diluent, and the stability of the diluted serum. A satisfactory procedure was as follows. The serum was separated by placing 125 gm of butter in centrifuge bottles and heating to 45° C in a boiling water-bath, centrifuging at 1700 r p m and siphoning off the fat. Two millilitres of the serum was diluted to 50 ml with 10% sodium acetate, the pH adjusted to 5-6 and the fluorescence determined immediately in a Coleman photofluorometer using a filter that transmitted light in the region of 365 mμ. This procedure gave fluorescence values that were correlated with flavour score ($r = - .84$) on salted butter stored at 32-2° C. (90° F.)

Introduction

Numerous objective methods for assessing the quality of butter have been investigated without marked success. These include measurements of the aldehyde (9), peroxide (7), and free fatty acid (2) contents of the fat; and titratable acidity, hydrogen ion concentration, and amino nitrogen content of the serum (2). The measurement of fluorescence has been applied to a variety of foodstuffs (3, 4, 5) to assess the changes induced by storage. Concomitant organoleptic assessment of the products has shown marked relation between flavour deterioration and fluorescence values for some materials, e.g., powdered eggs, while for others the objective test is not at all indicative of flavour status. This is to be expected since, for the materials studied, fluorescence is an attribute of the salt extract of the defatted material and as such primarily reflects changes in the non-fat components. Increasing the moisture content of egg powder and ration biscuits (8, 3) brought about an increase in fluorescent material, and in the latter suppressed oxidative changes in the fat. These observations suggested that the serum separated from butter would contain fluorescing substances in amounts that increase as the butter deteriorates. This paper describes the factors affecting the measurement of fluorescence in fresh and spoiled salted butter, and demonstrates the changes in fluorescence value under accelerated storage conditions.

Procedure

Preliminary Trials

After several trials it was observed that serum and butter fat could be separated satisfactorily by heating 125 gm of butter at 60° C. for five minutes

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and centrifuging for 10 min. at 1700 r.p.m. The fat was removed by siphoning and the clearest portion of serum pipetted into an Erlenmyer flask.

One millilitre of serum was diluted to 20 ml. with one of the solutions to be described, thoroughly mixed, and passed through No. 1 Whatman filter paper. The fluorescence of a 15 ml. portion of the filtrate was determined by means of a Coleman photofluorometer with standard 'Vitamin B₁' filters transmitting active light of wave-length 365 m μ . The photofluorometer was standardized by adjusting the instrument to give a scale reading of 50 for a solution containing 0.20 γ of quinine sulphate per ml. (5).

Effect of Diluents

During preliminary trials it was observed that when sera from fresh and spoiled samples of salted butter were diluted with 10% potassium chloride solution their fluorescence values differed by 38 photofluorometer units. These sera were turbid, presumably owing to dispersion of fat particles. It was believed that fat solvents mixed with other diluents for the serum might eliminate this difficulty. However, mixtures of dioxane, acetone, and chloroform with various salt solutions proved unsatisfactory. A number of diluents when used by themselves showed promise and merited further investigation.

An aqueous solution of sodium acetate was found to be the most satisfactory solvent because it gave the greatest difference in fluorescence readings between fresh and spoiled butter (viz., 45.0 and 65.6, respectively) and the solution was only slightly turbid. The sodium chloride, ammonium chloride, and ethyl alcohol solutions gave satisfactory differences but were too turbid. Sodium acetate, sodium chloride, ethyl alcohol solutions, and water were selected for further study.

Effect of pH

Hydrogen ion concentration has been shown to have an effect on the fluorescence of an extract of defatted dried egg powder (6). In the present study, pH effects were evaluated using the selected diluents mentioned above. Water and solutions of sodium chloride and ethyl alcohol were adjusted to the desired pH by adding dilute hydrochloric acid or sodium hydroxide solution; the pH of the sodium acetate solution was adjusted with dilute acetic acid or sodium hydroxide.

The diluted sera from spoiled butter increased in turbidity with increase in pH between 4 and 9, while that from fresh butter remained fairly clear. At pH 2 all the diluted sera were quite clear but fluorescence values were small. Sera, diluted with sodium acetate solution adjusted to pH 5, resulted in the clearest extracts, and a large difference in fluorescence values between fresh and spoiled butter (viz., 16.0 and 87.0, respectively). Therefore, a 10% solution of sodium acetate adjusted to pH 5-6 was selected as an appropriate diluent and used in all subsequent work.

Effect of Temperature

It was observed in the above study that duplication of results was poor in some instances. Since it had been demonstrated that temperature affected

the fluorescence of extracts of dried whole egg powder (6), it was considered that temperature variations might be responsible for the difference between duplicates. To evaluate this the procedure was modified as follows: samples of butter placed in centrifuge bottles and heated in a boiling water-bath were centrifuged when the samples were at each of the following temperatures: 35°, 40°, 50°, 60°, and 80° C. Fluorescence values were determined for each sample as previously described.

The results showed that temperature had little effect on the fluorescence of sera from fresh butter, but an increase in temperature caused a small decrease in fluorescence of sera from spoiled butter. The greatest difference in fluorescence values between fresh and spoiled butter (*viz.*, 26.0 and 35.0, respectively) was obtained for sera separated by heating to 40° or 50° C. Hence heating to 45° C. in a boiling water-bath prior to centrifuging was believed desirable.

Effect of Dilution

The fluorescence of a solution may be quenched by various factors, such as too great a concentration of fluorescing substance, which may be avoided by proper dilution. Such procedures may introduce other errors, namely, quenching or apparent quenching due to the solvent or instrument error (10). Therefore, it was of value to determine behaviour of butter sera diluted to various concentrations.

One-millilitre aliquots of serum, obtained from a sample of spoiled butter, were diluted to the following volumes with 10% sodium acetate solution (pH 5.6): 20, 30, 40, 50, 60, 70, 90, 100, and 200 ml. Fluorometric readings on these solutions are shown in Fig. 1. At the higher concentration there is slight quenching, as the relation deviates from the linear. Therefore solutions should be diluted so that readings will fall between 10 and 70 fluorescence units.

Stability of Diluted Sera

The time elapsing between dilution of a serum and photofluorometric measurement might conceivably affect the fluorometric value obtained. To study this, serum obtained from spoiled butter was diluted 1 : 50 with 10% sodium acetate of pH 5-6, allowed to stand at room temperature, and fluorometric values determined at intervals throughout a six-hour period. The data show that the diluted serum is reasonably stable at room temperature, since there is a decrease of only five fluorometer units (*viz.*, 45.0 to 40.0) in six hours.

Recommended Procedure

As a result of the above observations the following procedure was adopted as being most suitable. Whole salted butter (125 gm.) weighed into a centrifuge bottle was heated to 45° C. with constant stirring in a boiling water-bath,

and centrifuged for 10 min. at 1700 r.p.m. Fat and serum were separated by centrifuging and siphoning off the fat. Two millilitres of clear serum was diluted to 50 ml with 10% sodium acetate solution (pH 5-6), mixed, and filtered through No. 1 Whatman filter paper. The fluorometric value of the

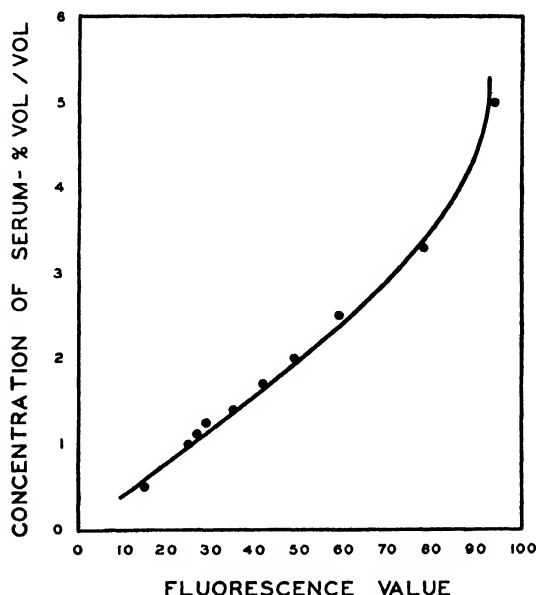


FIG 1 *Effect of dilution on the fluorescence value of spoiled butter serum.*

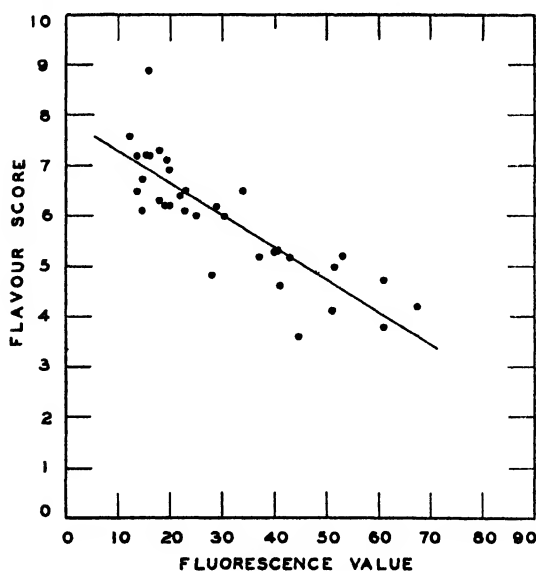


FIG. 2. *Relation between flavour score and fluorescence values on samples of stored butter.*

filtrate was determined in a Coleman photofluorometer. As the diluent had a small fluorescence value it was necessary to correct all fluorescence readings.

Evaluation of the Method on Stored Butter

Materials and Methods

The material investigated consisted of four sets of samples of canned and printed salted butter from an eastern and a western Canadian creamery. The butter was stored at 32.2° C. (90° F.) for 32 days and sampled at intervals to give a wide range of quality. To assess the suitability of the fluorometric method, fluorescence values were compared with flavour scores. The usual method for scoring butter was not employed as it is not readily adaptable to statistical treatment. Butter was scored as follows: 10, excellent; 8, good; 6, fair; 4, poor; 2, bad; 0, inedible. The ten tasters were required to score a set of four samples chilled to approximately 10° C. (50° F.).

Results

Fluorescence values increased with a decrease in flavour score (Fig. 2). Good agreement is indicated between the two ($r = -.84$). The equation for these data is:

$$y = 7.867 - 0.0632 x,$$

where x = corrected photofluorometer readings and y = flavour score. It is evident that fluorescence values of 30 and 61 correspond to flavour scores of 6 and 4, respectively.

Statistical analyses of the data obtained for each sample of butter showed a high correlation and no difference between regression coefficients. There was no significant increase in correlation by using the log of fluorescence values, which is the usual form of curve to be expected. However, this may possibly be due to insufficient samples of low and high flavour scores. The co-linearity of the four sets of data was slightly different. While this difference was statistically significant it is probable that the taster level of scoring did not remain constant.

Discussion

The high correlation and lack of significant difference in regression coefficients between fluorescence measurements and flavour scores indicate that this test should be a valuable aid in assessing the keeping quality of butter under unfavourable storage temperatures. However, this test will not assess flavour deterioration in butter due to tainting by foreign materials, nor can it be definitely stated whether it will apply to other forms of spoilage that may occur in commercial practice (1, pp. 75-92).

Acknowledgments

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THE BACTERIAL FLORA OF LOW-ACID VEGETABLES CANNED AT 212° F.

II. A PRELIMINARY STUDY OF THE EFFECTS OF EXTRACTS FROM PROCESSED TOMATO JUICE AND FROM TOMATO PLANTS ON BACTERIA INVOLVED IN FOOD PRESERVATION¹

J. W. CONNER²

Abstract

Commercially processed tomato juice, dehydrated tomato stems, leaves, seed, and fruit (variety, Sutton's Very Earliest), and tomato seedlings (variety Pan America), were extracted with methanol and the extracts tested for antibacterial properties against certain species of bacteria important in the food industries, and other Gram-positive and Gram-negative types. The extracts prepared from tomato juice and tomato fruit inhibited the growth of most of the test organisms.

Introduction

The fact that certain plant extracts possess antibacterial properties has been known for some time. Burkholder (1) reported that extracts of 27 species of lichens possessed antibacterial properties. C. S. Pederson and P. Fisher (6) found that the expressed juices of certain cabbages inhibited the growth of *Leuconostoc mesenteroides*, *Lactobacillus plantarum*, and *Lactobacillus brevis*. In 1944, Lucas and Lewis (5) reported antibacterial principles in the expressed juices of *Onopordon acanthium* (Scotch thistle), *Verbascum thapsus* (common mullein) and *Paeonia officinalis* against *Staphylococcus aureus*. The same authors also found antibacterial principles in some varieties of *Lonicera tatarica* (one of the honeysuckles) active against both *Staphylococcus aureus* and *Escherichia coli*.

The discovery by Irving, Fontaine, and Doolittle (3) of 'lycopersicin', a fungistatic agent from the tomato plant, suggested that the tomato juice used in processing low-acid vegetables (1) might contain an antibiotic agent capable of suppressing the growth of certain micro-organisms. Irving, Fontaine, and Doolittle (3) reported that 'lycopersicin' inhibited the growth of *Fusarium oxysporum* (Fusarium Wilt) when tested by the cylinder-plate method.

In the light of these discoveries, extracts of canned tomato juice and of tomato plants at various stages of growth were prepared and tested against certain species of bacteria important in the food industry, and other Gram-positive and Gram-negative types.

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Methods

It was originally intended to isolate from tomato juice a material similar to that obtained by Irving, Fontaine, and Doolittle (3) from Pan America and other varieties of tomato plants. Their technique (3), with certain necessary modifications, was followed in the isolation of the active principle. Untreated, filtered, and dehydrated samples of commercially processed tomato juice were examined. Methanol extracts of the leaves, stems, seeds, and fruit (green, partially ripe, and ripe) of mature tomato plants (variety, Sutton's Very Earliest), and of tomato seedlings (variety, Pan America) were prepared, and the extracts evaporated to dryness. The residue was taken up, in some instances with sterile distilled water, and, in others, with sterile phosphate buffer solution. In a number of experiments with tomato juice filtrates, the pH was raised to 5.40 and 7.08 prior to extraction. The chemicals used in the extractions were tested and did not show antibacterial activity.

The aerobic test organisms used were *Bacillus thermoacidurans* (A.T.C.C. No. 8038), *Bacillus subtilis* (Penicillin resistant), *Bacillus subtilis* (penicillin sensitive), *Escherichia coli*, *Eberthella typhosa*, *Lactobacillus lycopersici* (A.T.C.C. No. 4005), *Salmonella aertrycke*, *Salmonella anatis*, *Salmonella enteritidis*, *Salmonella morgani*, *Salmonella paratyphi*, *Salmonella psittacosis*, *Salmonella schottmuelleri*, *Salmonella typhimurium*, *Serratia marcescens*, *Shigella dysenteriae*, *Shigella flexneri*, *Shigella gallinarum*, *Shigella sonnei*, *Staphylococcus aureus*, and *Staphylococcus citreus*. The anaerobic bacteria tested were *Clostridium butyricum*, *Clostridium thermosaccharolyticum* (A.T.C.C. No. 7956), and *Clostridium sporogenes* (A.T.C.C. No. 3679). All the trial organisms were not used with the various extracts, owing to the small amount of extract available. With the aerobes, the cylinder-plate method was employed in testing the extracts and a modification of the thio-glycollate agar dilution technique of Lock (4) was used for the anaerobes.

Results

Untreated and Filtered Tomato Juice

Preliminary experiments with untreated tomato juice and tomato juice filtrate employing the cylinder-plate technique were conducted. Both the plain tomato juice and the filtered juice inhibited the growth of *S. citreus*, producing zones of inhibition measuring 13 mm. in diameter. Very little, if any, inhibition of the growth of *B. subtilis* was evidenced. However, the organisms did not grow directly under the liquid in the cylinders.

Dehydrated Tomato Juice and Pulp

Commercially processed whole tomato juice and pulp filtered from tomato juice, dehydrated at 55° C., yielded extracts possessing antibacterial properties against several species of bacteria. The results are presented in Table I. The extracts were tested by the cylinder-plate technique. This crude extract inhibited the growth of the test organisms when used undiluted and when diluted 1 to 1 or 1 to 2 with sterile distilled water or with sterile phosphate

TABLE I

AVERAGE DIAMETERS OF ZONES OF INHIBITION PRODUCED BY EXTRACTS FROM DEHYDRATED TOMATO JUICE AND PULP

Test organisms	Zones of inhibition, mm		
	Not diluted	Diluted 1 : 1	Diluted 1 : 2
<i>B. subtilis</i> (penicillin resistant)	29.5	25.0	16.0
<i>B. subtilis</i> (penicillin sensitive)	21.0	19.0	11.0
<i>E. coli</i>	21.3	17.5	16.0
<i>E. typhosa</i>	32.0	28.0	18.5
<i>L. lycopersici</i>	21.5	14.0	Nil
<i>S. aertrycke</i>	37.0	29.0	19.0
<i>S. anatis</i>	21.0	17.0	11.0
<i>S. enteritidis</i>	24.0	17.0	12.5
<i>S. morgani</i>	25.0	21.0	14.0
<i>S. paratyphi</i>	18.5	18.0	12.5
<i>S. psittacosis</i>	37.5	28.5	21.5
<i>S. schottmuelleri</i>	20.0	16.5	9.5
<i>S. typhimurium</i>	21.0	19.5	Nil
<i>S. marcescens</i>	18.5	10.5	9.0
<i>S. dysenteriae</i>	32.0	28.0	24.0
<i>S. gallinarum</i>	25.0	21.5	18.5
<i>S. flexneri</i>	40.5	32.0	21.0
<i>S. sonnei</i>	30.0	23.5	16.5
<i>S. aureus</i>	30.5	25.5	14.0
<i>S. citreus</i>	42.6	32.5	29.0

buffer. The zones of inhibition decreased with dilution of the extract. It is interesting to note that the phosphate buffer alone inhibited the strain of *S. citreus* used, producing a zone of inhibition measuring 22 mm. in diameter. The other organisms were not affected by the buffer solution.

Tomato Juice Filtrate

Several extractions were prepared using commercially processed tomato juice, which was filtered through filter paper. The diameters of the zones of inhibition produced against the test organisms by these crude filtrates are presented in Table II. In general, when the pH of the filtered juice was raised to 5.40 and 7.08, and when the phosphate buffer was employed as the medium for dissolving the principle, the zones of inhibition produced were more extensive than those produced by the principle dissolved from the final residue in sterile distilled water. These results are presented in Table III. Since most of the extractions were qualitative, it is difficult to compare accurately separate extractions. However, it would appear from Tables I and II that the extracts prepared from dehydrated juice were more active than those from the filtrate.

The growth of the same test organisms was inhibited by filtrate and dehydrated juice extracts. In most cases, a zone of increased growth surrounding the clear zone of inhibition appeared on the plates. In greater dilutions the extract apparently acted as a stimulant to bacterial growth. This phenomenon is particularly noticeable with *S. marcescens*. A typical

TABLE II

AVERAGE DIAMETERS OF ZONES OF INHIBITION PRODUCED BY EXTRACTS FROM TOMATO JUICE FILTRATE

Test organisms	Zones of inhibition, mm.		
	Not diluted	Diluted 1 : 1	Diluted 1 : 2
<i>B. subtilis</i> (penicillin resistant)	20.5	16.5	14.5
<i>B. subtilis</i> (penicillin sensitive)	34.0	22.0	19.0
<i>B. thermoacidurans</i>	12.0	9.0	Nil
<i>E. coli</i>	19.0	14.5	10.0
<i>E. typhosa</i>	25.0	23.0	22.0
<i>L. lycopersici</i>	29.5	15.0	14.0
<i>S. aertrycke</i>	19.5	14.0	10.0
<i>S. anatis</i>	21.0	19.0	17.5
<i>S. enteritidis</i>	29.5	22.0	20.5
<i>S. morgani</i>	20.5	18.0	14.5
<i>S. paratyphi</i>	26.0	23.0	20.0
<i>S. psittacosis</i>	27.0	25.0	19.0
<i>S. schottmuelleri</i>	25.0	18.0	20.5
<i>S. typhimurium</i>	27.0	23.0	17.5
<i>S. marcescens</i>	14.0	10.5	9.0
<i>S. dysenteriae</i>	25.5	19.5	17.5
<i>S. gallinarum</i>	21.5	18.0	15.0
<i>S. flexneri</i>	20.0	19.0	16.0
<i>S. sonnei</i>	17.0	14.0	12.5
<i>S. aureus</i>	15.5	15.0	9.0
<i>S. citreus</i>	27.5	24.0	23.0

TABLE III

AVERAGE DIAMETERS OF ZONES OF INHIBITION PRODUCED BY VARIOUS EXTRACTS FROM TOMATO JUICE FILTRATE

Organisms	pH prior to extraction	Diluent	Zones of inhibition, mm.	
			Undiluted	Diluted 1 : 1
<i>B. subtilis</i>		Phosphate buffer	31.0	25.0
	5.40	Sterile distilled water	16.5	14.0
	7.08	Sterile distilled water	14.0	10.0
<i>E. coli</i>		Phosphate buffer	34.6	23.6
	5.40	Sterile distilled water	13.0	10.0
	7.08	Sterile distilled water	11.0	10.0
<i>S. marcescens</i>		Phosphate buffer	18.3	10.5
	5.40	Sterile distilled water	13.0	—
	7.08	Sterile distilled water	12.0	11.0
<i>S. aureus</i>		Phosphate buffer	33.3	25.3
	5.40	Sterile distilled water	13.0	11.0
	7.08	Sterile distilled water	10.0	9.0
<i>S. citreus</i>		Phosphate buffer	37.3	27.6
	5.40	Sterile distilled water	24.0	—
	7.08	Sterile distilled water	28.0	26.0
		Sterile distilled water	24.0	19.0

example shows a clear zone of absolute inhibition measuring 13 mm. in diameter. Surrounding this zone, and covering a surface area of 1 mm. diameter, was a zone of moderate growth, followed by a 9 mm. zone of extremely heavy, raised, moist, and shiny growth. Beyond this, a 7 mm. zone of less than average plate growth appeared, merging gradually to the average amounts of growth on the agar, distant from the cylinder. With *S. citreus*, a plate showing a clear zone of 29 mm. possessed a 5 mm. zone in which the growth was very heavy and then average growth over the remainder of the plate.

Tomato Plants

The seeds, roots, stems, and leaves of tomato plants (variety, Sutton's Very Earliest), grown under glass, did not, under the conditions of these experiments, possess an appreciable amount of the active principle obtained from the tomato juice and fruit. The only exception was one extract prepared from a mixture of dehydrated leaves and stems, which produced a very small zone of inhibition with *S. marcescens*.

The results obtained with extracts from tomato seedlings (variety, Pan America) were inconclusive. One extract failed to inhibit the growth of *B. subtilis*, *Bacillus stearothermophilus*, *B. thermoacidurans*, *E. coli*, *S. aureus*, *S. citreus*, and *S. marcescens*. Another extract, also from Pan America seedlings, produced zones in which the inhibition of growth was doubtful with *B. subtilis*, *E. coli*, *S. citreus*, and *S. marcescens*.

Tomato Fruit

Thinly sliced tomato fruit (variety, Sutton's Very Earliest) was dried at 55° C. Methanol extracts of the dry residue contained the active principle. The first extract prepared from the partly mature tomatoes was tested on only three organisms, because of the small amount of buffered extract available. The extract was almost twice as active against the spore-forming, Gram-positive rod, *B. subtilis*, as it was against *S. aureus* and *S. marcescens*. This inhibition of *B. subtilis* is in contrast to the very slight inhibition obtained in the preliminary experiment using untreated tomato juice. Further tests with extracts prepared from ripe, partly ripe, and green fruits on several species of bacteria gave results comparable with those obtained with the tomato juice extracts. The extracts prepared from ripe fruits seemed more active than those obtained from partly ripe and green fruits (Table IV). In high dilutions, the extract from ripe tomatoes prevented the growth of *C. butyricum*, *C. sporogenes*, and *C. thermosaccharolyticum*. In most tests, when 3 ml. of the extract was diluted with 4 ml. of thioglycollate agar, these organisms failed to grow. In lower dilutions the extract stimulated growth, and with *C. butyricum* and *C. sporogenes* a larger amount of gas was produced than with the plain thioglycollate agar. (Plates I, II, and III show the inhibition of the growth of some bacteria by extracts from tomato fruit).

TABLE IV

AVERAGE DIAMETERS OF ZONES OF INHIBITION PRODUCED BY EXTRACTS FROM TOMATOES

Test organisms	Zones of inhibition in mm		
	Undiluted	Diluted 1 : 1	Diluted 1 : 2
A* <i>B. subtilis</i> (penicillin resistant)	19 0	18 0	16 0
<i>B. thermoacidurans</i>	9 0	—	—
<i>B. stearothermophilus</i>	20 0	16 0	9 0
<i>E. coli</i>	25 0	22 0	13 0
<i>S. aureus</i>	22 0	23 0	21 0
<i>S. citreus</i>	36 5	30 0	22 0
<i>S. marcescens</i>	9 0	Nil	Nil
B† <i>B. subtilis</i> (penicillin resistant)	24 0	19 0	15 5
<i>B. thermoacidurans</i>	12 0	—	—
<i>B. stearothermophilus</i>	20 0	19 0	10 0
<i>E. coli</i>	27 0	11 0	—
<i>S. aureus</i>	20 0	14 0	10 0
<i>S. citreus</i>	26 0	17 5	20 5
<i>S. marcescens</i>	15 5	13 5	12 0
C‡ <i>B. subtilis</i> (penicillin resistant)	29 0	24 5	18 0
<i>B. thermoacidurans</i>	10 0	Nil	Nil
<i>B. stearothermophilus</i>	19 0	12 0	10 0
<i>E. coli</i>	17 5	14 0	10 0
<i>S. aureus</i>	21 0	21 0	18 5
<i>S. citreus</i>	19 0	14 0	11 0
<i>S. marcescens</i>	16 5	12 5	Nil§

* Extract from green tomatoes

† Extract from partly ripe tomatoes

‡ Extract from ripe tomatoes

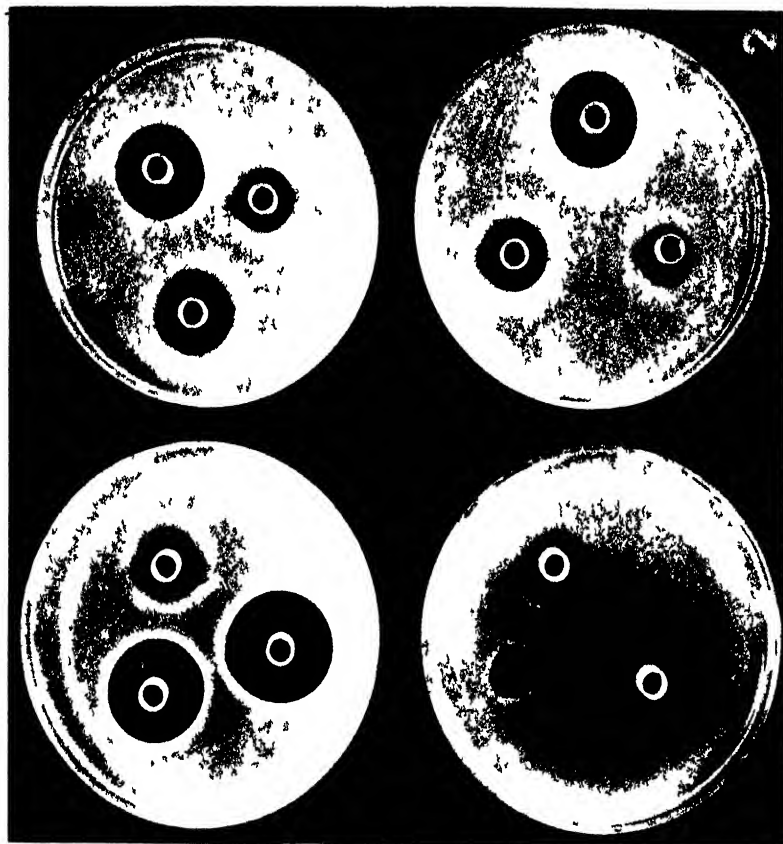
§ 5 mm zone of heavy growth around cylinder with no pigment

Discussion

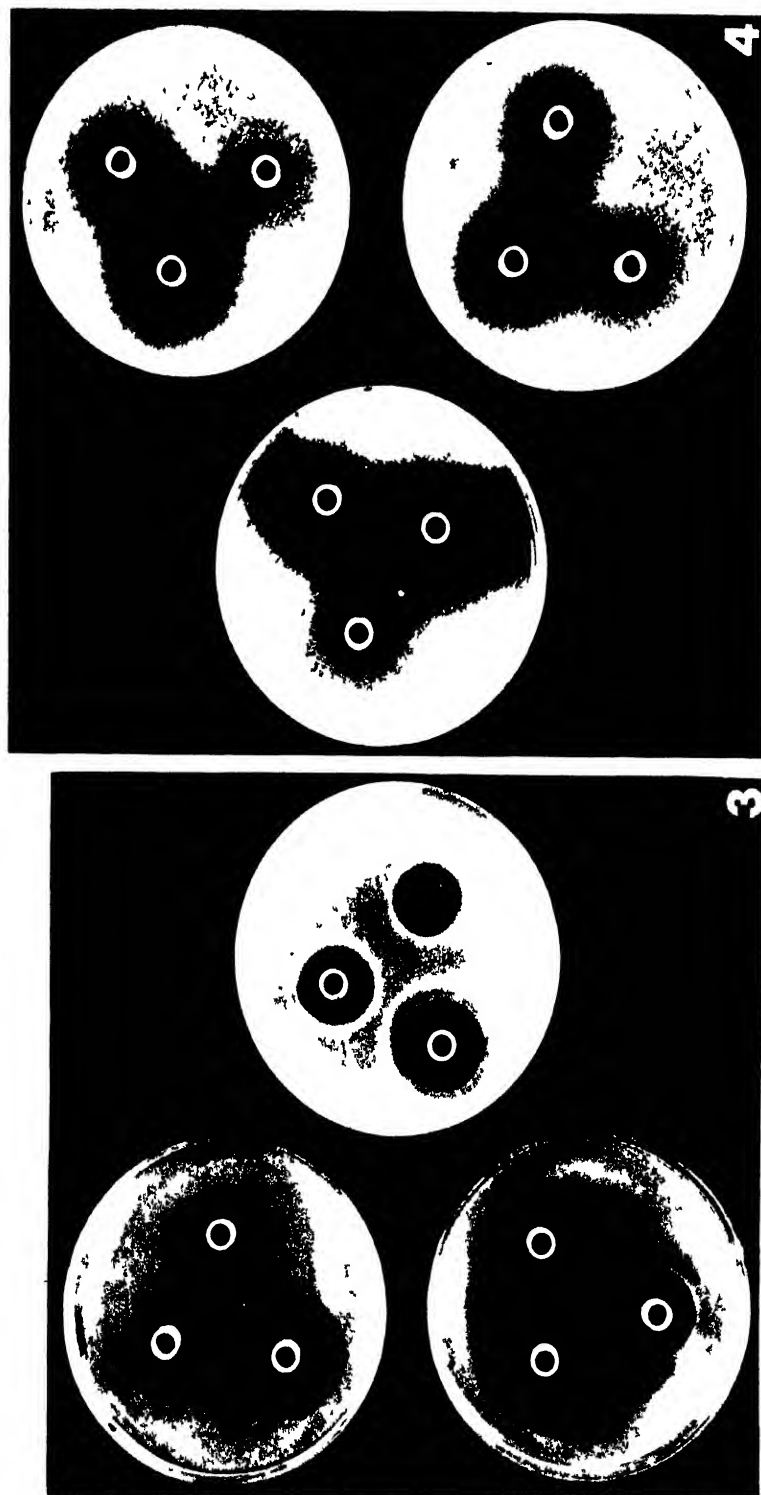
It seems evident from these preliminary experiments that tomatoes contain a principle capable of antibiotic action. The principle obtained by the methanol extraction is active *in vitro* against both the Gram-positive and Gram-negative bacteria tested.

The principle is present mainly in the fruit and is not injured by the commercial processing of tomato juice. The presence of the principle in the seed, leaves, stems, or roots of tomato plants (variety, Sutton's Very Earliest) grown under greenhouse conditions could not be demonstrated. This would indicate that the principle obtained in this laboratory differed from 'lycopersicin.' Irving, Fontaine, and Doolittle (3), reported lycopersicin, a fungistatic agent from tomato plants, as being present in the stems, roots, and leaves, but not in the fruit or seeds.

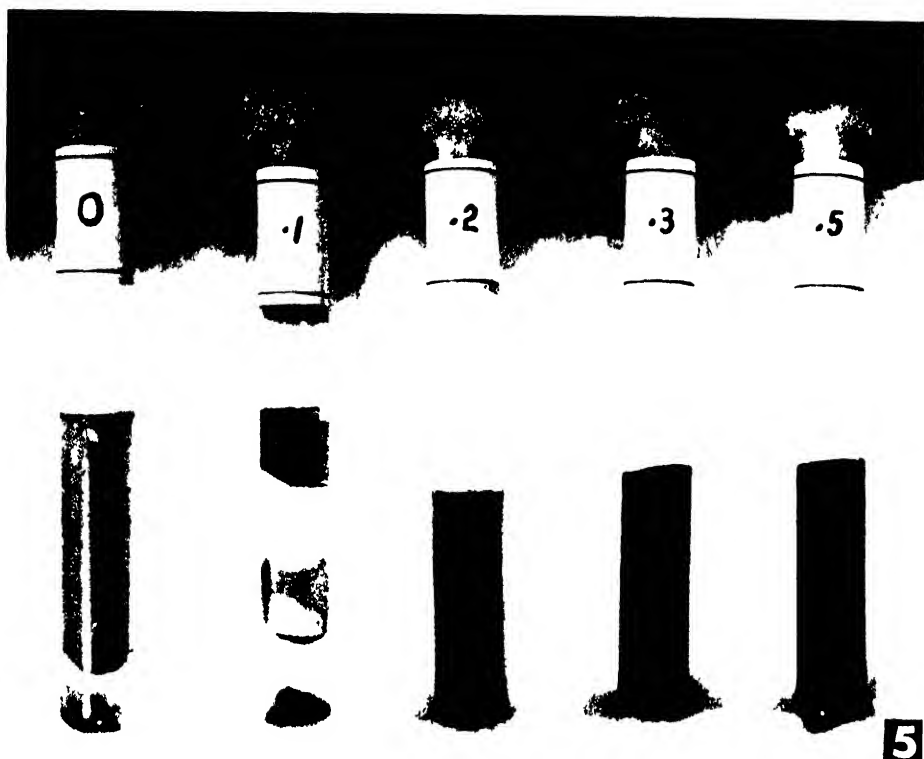
It seems probable that the lower surviving bacterial population obtained by the addition of 50% tomato juice to the covering solution in the processing¹ of low-acid vegetables at 212° F. (2), might be due, at least in part, to an active bacteriostatic principle originating in the tomato fruit.



FIGS 1 and 2 Cylinder-plate assays of extracts from tomatoes FIG 1 Control plate inoculated with *B. subtilis* (penicillin resistant), and cylinders filled with sterile phosphate buffer FIG 2 Plates inoculated with *B. subtilis* and cylinders filled with extract (undiluted, diluted 1:1, and diluted 1:2) from tomato fruit (variety, Sutton's Very Earliest) and seedlings (Pan America) Upper left, extract from ripe fruit; upper right, extract from partly ripe fruit, lower right, extract from green fruit, and lower left, extract from tomato seedlings



FIGS. 3 and 4. Cylinder plate assays of extracts from tomatoes. FIG. 3. Plates inoculated with *E. coli* and cylinders filled with extract (undiluted, diluted 1:1, and diluted 1:2) from tomato fruit. Upper left, extract from partly ripe fruit; lower left, extract from partly ripe tomatoes; lower right, extract from ripe tomatoes. (Note overgrowth on prolonged incubation with the extract diluted 1:2.)



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FIG. 5 Effect of extract from ripe tomatoes on *C. sporogenes* inoculated into thioglycollate agar containing various amounts of the extract. 0.2 ml. of the extract mixed with 4 ml. of agar inhibited the growth of this test organism.

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THE DYEING OF NATURAL COTTON WITH DIRECT DYES: SOME EFFECTS DUE TO TEMPERATURE, DYE CONCENTRATION, AND ANIONIC SURFACE-ACTIVE AGENTS¹

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Abstract

The presence of an anionic surface-active agent in the dyeing of natural cotton yarn with a purified direct dye, using a dye-bath containing sodium chloride, is shown to increase the rate of dyeing, and to increase the amount of dye sorbed at equilibrium. The rate-accelerating effect has been studied as a function of the concentration of surface-active agent, using both commercial materials and methanol-extractable fractions of the latter. The effect exerted by an anionic surface-active agent, both on the equilibrium sorption and on the rate of dyeing, decreases as the temperature is increased. An explanation of the data, in terms of an interaction between the fibre and surface-active agent, is advanced.

Studies carried out in the absence of a surface-active agent show that with increased temperature of dyeing the rate of dye sorption is increased, but the value of the equilibrium sorption is decreased; the dyeing process is exothermic. The relation between equilibrium sorption and residual dye-bath concentration is expressible by a Freundlich equation, and that between equilibrium sorption and initial dye-bath concentration is linear, at least over the range of concentration studied. The time required to reach a state of equilibrium increases as the initial concentration of dye in the bath is increased.

Introduction

The discovery in 1884 that Congo Red was a substantive dye for cotton led to the development of a very large number of synthetic direct cotton dyes. The application of these dyes to cellulosic materials has been extensively studied since the late nineteenth century, but, as has been pointed out in an excellent recent review (35) of the literature of the dyeing of cellulose with direct dyes, "it is only since 1933 that the investigations have been placed on a sound experimental basis." Prior to 1931 satisfactory rapid methods for the purification of commercial direct dyestuffs were not available, and, also, in many of the early studies the distinction between equilibrium dyeing and the rate of attainment of equilibrium was not always clearly made. Within about the last decade, however, a number of valuable studies, for example, of the effect of temperature and inorganic electrolytes on the equilibrium dye sorption and the rate of its attainment, and of the diffusion coefficients of direct dyes in water and into cellulose have been carried out using purified dyes, and important contributions relative to the mechanism of the dyeing process have been published (see, for example, the review papers by Standing (35), Boulton and Morton (2), and Neale (21) and recent papers by these

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² Contribution from the Department of Chemistry, McMaster University, Hamilton, Ont. This paper was presented before the Division of Textile Chemistry at the Canadian Chemical Conference on June 25, 1946.

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workers and their collaborators). Most of the careful experimental work with cellulosic material has been carried out using regenerated cellulose—viscose yarn or viscose (cellophane) sheet.

In recent years some work involving the interaction of direct dyes and cotton has been reported, viz., comparisons of equilibrium dye sorption on bleached cotton cloth with that on viscose sheet (6, 8, 10, 21, 26) and on other forms of cellulose (10, 25), the effect of salt concentration on the equilibrium sorption of dyes on bleached cotton cloth and other cellulosic materials (6, 10, 26), studies of equilibrium dye sorption on bleached cotton cloth from a mixed dye-bath (28) and on cotton yarn previously dyed with a vat dye (27), a comparison of the rate and extent of dye sorption on cotton hairs and viscose yarn (2), the effect of temperature on the equilibrium sorption of certain direct dyes on cotton (6, 12), the effect of the ash content of cotton on its dye sorption (12), the dependence of certain dyeing properties on the source of the cotton (37), and the desorption of direct dyes from cotton (11, 13, 14, 32, 40). The comment was made (2) in 1940 that "the investigation of the natural cellulosic fibres, cotton, mercerized cotton . . . , along the lines which have proved so fruitful with regenerated cellulose, has been surprisingly neglected"; only a portion of the aforementioned work with cotton has appeared since this view was expressed, and the statement remains essentially true in 1946.*

In the present work, the dyeing of natural cotton yarn has been studied using a purified blue direct dye. The effects on the equilibrium sorption of dye and the rate of its attainment of varying the temperature of dyeing, of varying the dye concentration, and of adding an anionic surface-active agent to the dye-bath, have been studied.

Surface-active agents are widely used as dye-bath assistants in the direct dyeing of cotton; their use has been recommended, for example, to facilitate the 'wetting-out' of cotton in the dye-bath and to promote level dyeing. The effect of anionic surface-active agents on the sorption of direct dyes by cellulosic material has received, as will be noted later, only scant attention.

Materials and Methods

Cotton

Natural cotton yarn of uniform quality was obtained from Canadian Cottons Ltd., Hamilton, Ont.; the yarn was $\frac{1}{4}$'s count wound on Franklin springs in 14 oz. packages.

For experimental dyeings, the cotton yarn was wound, using a miniature mechanical skein winder, into skeins weighing approximately 5.5 gm., dried at 110° C. (15, pp. 462-464) for 24 hr. (constant weight was attained at this temperature in roughly half this time), and cooled in a desiccator over phosphorus pentoxide. The sample of yarn was weighed rapidly, and sufficient

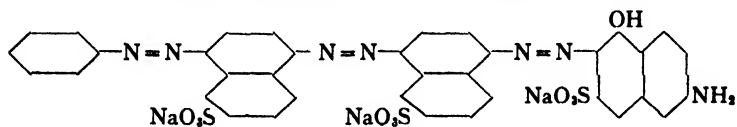
* It has been pointed out (20) that the interpretation of dyeing experiments with cotton yarns is more difficult than the interpretation of those in which viscose sheet is used because of the embedding of the fibres in yarn.

cut from the skein to bring the weight to about 5.02 gm. The yarn was then dried for a further six hours at 110° C., cooled as before, weighed rapidly, and the weight adjusted to 5.000 ± 0.002 gm. by cutting. Such a procedure was adopted in order to obtain an accurate dry weight.

Before being dyed, the weighed dry cotton skein was allowed to stand in air for 24 hr. to permit regain of moisture (15, pp. 462-464). The dry cotton yarn was found to be appreciably hygroscopic (the 'normal' moisture content will, of course, depend on the temperature and the relative humidity of the air).

Dyestuff

Calcodur Blue 4GL, after purification, was used as the dye throughout this work. This dye (Colour Index 533) is the sodium salt of benzene-azo-6-sulpho- α -naphthalene-azo-6-sulpho- α -naphthalene-azo-6-amino-1-naphthol-3-sulphonic acid ($C_{36}H_{22}N_7O_{10}S_3Na_3$, molecular weight: 877.8). The formula is:



The commercial dyestuff was purified in batches, by the following procedure, the principle of which is due to Rose (30). Forty grams of the commercial dyestuff was dissolved in a minimum quantity of hot (95° to 100° C.) water, and the solution filtered while hot. The hot filtrate was added to one litre of a solution containing 24 gm of di-*o*-tolylguanidine (Eastman Kodak product) and 25 ml of 12 *M* hydrochloric acid. The mixture was allowed to cool, and the heavy dark precipitate was filtered and washed repeatedly with water. After the filter cake had been sucked to 'dryness' it was dissolved in methanol (synthetic, 99.85%), and to this solution was added a quantity of a 2 *M* solution of sodium hydroxide in methanol calculated to be sufficient to precipitate (as the sodium salt) about half the dye present. After two days, the precipitated dye was filtered, washed with methanol, dried at 110° C., and ground to a fine powder. The several batches were thoroughly mixed. Before preparing solutions, the dye was dried in an oven at 110° C. for 24 hr., and cooled in a desiccator over phosphorus pentoxide (the purified dye was appreciably hygroscopic, as were also other direct dyes (22)).

The absorption spectrum of the purified dye (in the range 350 to 650 m μ) was detectably different from that of the commercial dyestuff, but subjection of a batch of purified dye to a second similar purification procedure did not result in any further change in the absorption spectrum. The spectrum of a partially exhausted (using cotton yarn) dye-bath solution was compared with that of a dye solution diluted to approximately the same concentration as the partially exhausted bath, and the two found to be almost identical. This may be taken to mean (21, 35) that the purified dye was essentially free from any coloured impurities differing in their substantivity on cotton from that of the main dye.

By means of spectrophotometric tests, aqueous solutions of the dye stored in soft-glass and in Pyrex containers, in diffuse and in direct sunlight and in the dark, were found to be stable, at least over a period of one week (solutions tested daily). Another dye solution, stored in diffuse sunlight indoors, was examined in the spectrophotometer every few hours for a period of two days; no evidence of instability was found.

Surface-active Agents

A representative group of anionic surface-active agents were obtained through the courtesy of a number of industrial concerns. Some of these products were stated by the manufacturer to be 100% 'active', others were stated to contain various amounts of inorganic salts (commonly sodium sulphate).

As stated below, certain experiments were carried out with the commercial surface-active products; in others, samples purified by a methanol extraction process (31) were used. The extraction process was performed as follows. The product was treated in a Soxhlet extraction apparatus with methanol until the solvent siphoning from the extractor appeared colourless. The methanol solution of surface-active agent was then evaporated to about one-quarter of its volume and cooled, whereupon, in most cases, the agent crystallized from the solution. The crystallized product was dried in air at 110° C. The amount of material remaining in the thimble of the extractor varied from 0 to 65% of the weight of commercial product taken.

Dyeing Apparatus

The dye-pots were constructed from three-necked Woulff bottles of one litre capacity. Each bottle was cut in two (the cut was made about 1 in. below the necks, well above the line of the dye-bath solution) to allow the insertion of a stirrer; the cut edges were ground smooth to ensure a tight fit when sealed with cellulose tape and held together in a suitable clamping frame. The shaft of the stirrer operated through a sleeve arrangement in the centre neck of the flask; in one of the outer necks there was inserted a snug-fitting 'cold-finger' condenser, and in the other a ground glass plug that could be removed when samples of the dye liquor were taken for analysis.

Attention has been drawn by other workers (10) to the importance of suitable agitation of yarn in the dye-bath. The following design of stirrer was adopted after considerable experimentation: the shaft was of 6 mm. glass rod to which were attached, at the end, eight radiating spokes 30 mm. long of 6 mm. glass rod, with the terminals of alternate spokes formed into eyelets. The skein of cotton yarn was suspended beneath this circular stirring head by means of four pieces of platinum wire (B. & S No. 28) attached at one end to the glass eyelet and at the other to the centre of the shaft. This stirrer was given an up and down motion (stroke of 3 cm. and period of 1 sec.) in the dye-bath solution by means of an electromagnetic plunger-action device described elsewhere (38). This stirring device was found to give excellent results with respect to levelness of dyeing and reproducibility of results.

The dye-pots, equipped with requisite stirrers and condensers, were immersed in a constant temperature bath insulated so as to be capable of operation in the range 15° to 99° C., and equipped with a thermoregulator permitting temperature control to $\pm 0.1^{\circ}$ C. of the desired temperature.

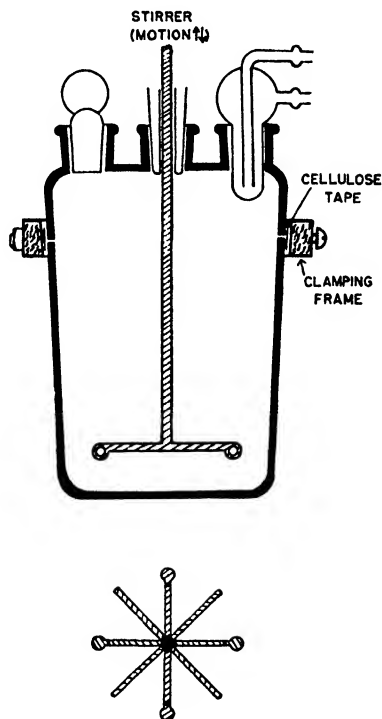


FIG. 1. Side view of dyeing apparatus (in full section) with bottom view of stirrer head shown underneath.

Dyeing Procedure

In all dyeings reported in this paper, $5\,000 \pm 0\,002$ gm. (dry weight) of cotton yarn was dyed using 250 ml. of dye solution. The dye solution, which always contained 4.00 gm. per litre of sodium chloride (analysed grade), varied in its initial concentration from 10.0 to 45.5 mgm. per litre (1.1×10^{-5} to 5.2×10^{-5} M).*

The assembled dye-pot, with the skein of cotton yarn in place, but not containing the dye-bath solution, was placed in the constant temperature bath and allowed to come to the appropriate temperature. In a Pyrex bottle, also contained in the bath, a solution containing dye, sodium chloride (and possibly a surface-active agent), at appropriate concentrations was brought to temperature. Using a pipette, 250 ml. of the dye-bath solution was removed

* Concentrations of dye-bath components are frequently expressed in terms of the weight of material being dyed. In these terms, the liquor-to-yarn ratio was 50 : 1, the salt concentration was 20%, and the dye concentration varied from 0.05 to 0.23%.

from the bottle, added to the dye-bath through the neck normally stoppered with a glass plug, and the stirrer and timer started.

At appropriately spaced intervals, small (5 ml.) samples of the dye-bath solution were removed through the neck of the dye-bath normally stoppered, cooled to room temperature, analysed spectrophotometrically (*vide infra*) for dye content, and then replaced in the dye-pot.

Measurement of Dye Concentration

Unknown concentrations of dye in solution were determined using a Coleman Model 11 Universal spectrophotometer, equipped with the 'PC-4' filter; matched rectangular cuvettes, with a path length of 5 mm., were employed. The reference cuvette contained distilled water. Calibration of the instrument (at a setting of 600 m μ , a maximum in the absorption spectrum) was effected at room temperature using dye solutions of known concentration (assuming that the dye was dry after heating in air for 24 hr. at 110° C.) and containing sodium chloride in the same concentration (4.00 gm. per litre) as used in dyeing experiments. The presence of the salt noticeably affected the spectrum—for example, changing the maximum from 610 to 600 m μ . Experiments showed that the transmittance (at least over the range 525 to 625 m μ) of a dye solution containing sodium chloride (4.00 gm. per litre) was not measurably affected, at room temperature, by the presence of surface-active agents in the concentrations encountered in this work.

A plot of the dye concentration against the logarithm of the per cent transmittance yielded a straight line, either in the presence or absence of sodium chloride (4.00 gm. per litre) with dye concentrations at least as high as 50 mgm. per litre ($5.7 \times 10^{-5} M$). This conformity with Beer's law would indicate that, at least up to this concentration, either the dye was not aggregated or else that the extent and nature of the aggregation remained constant.

From the concentration of dye remaining in the dye-bath liquor t minutes after the start of dyeing and the concentration of dye in the liquor before its contact with cotton (likewise determined with reference to the spectrophotometric calibration curve), the sorption* of dye on the cotton at time t may, of course, be readily calculated. Dye sorptions reported below are calculated in terms of milligrams of dye sorbed per 100 gm. of cotton yarn (dry weight). In the experiments below, surface-active agents were absent from the dye-bath liquor unless the contrary is stated.

Experimental Results

Variation in Initial Dye Concentration

A series of dyeings was carried out at 90° C. with the initial concentration of dye in the dye-bath solution varying from 10.0 to 45.5 mgm. per litre.

* Several workers (e.g., Neale, Boulton, and Standing) refer to the 'absorption' of direct dyes by or on cellulosic material. The term 'adsorption' is also sometimes employed. Inasmuch as these terms are used in different senses by different authors (see, for example, Thomas (36, pp. 272-273)), the present writers prefer the use of the non-committal term 'sorption' (17, 18).

The dye sorption as a function of time of dyeing is plotted in Fig. 2. The dyeing at an initial dye concentration of 20.0 mgm. per litre was carried out in duplicate; the results obtained (as well as data secured from other experiments) showed that the equilibrium sorption value was reproducible with a precision of within 1%.

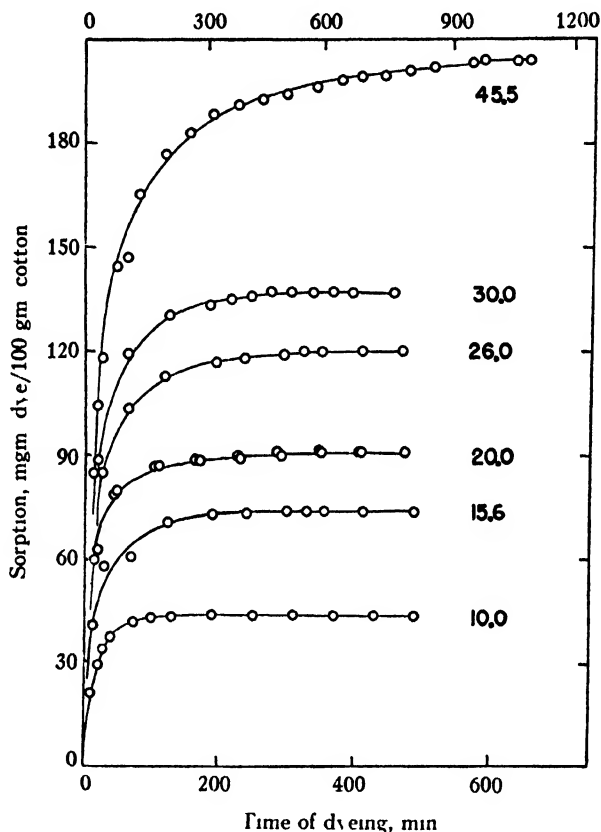


FIG 2 Effect of dye concentration on the equilibrium sorption and the rate of its attainment at 90° C. (The figures adjacent to the curves refer to initial dye concentrations in mgm per litre. The upper time scale applies only to the curve with an initial dye concentration of 45.5 mgm. per litre)

A plot of the logarithm of the equilibrium sorption value against the logarithm of the final, or equilibrium, concentration of dye in the bath is shown in Fig 3, and a plot of the equilibrium sorption value against the initial concentration of dye in the dye-bath is given in Fig 4

Variation in Temperature

A number of dyeings were carried out, over a range of temperature, with an initial dye concentration of 26.7 mgm. per litre. The resultant data are plotted in Fig 5

Sorption-time curves were also obtained at 80° and 90° C. for dyeings in which the initial dye concentration was 20.0 mgm. per litre. The data obtained in these runs are plotted semilogarithmically in Fig. 6.

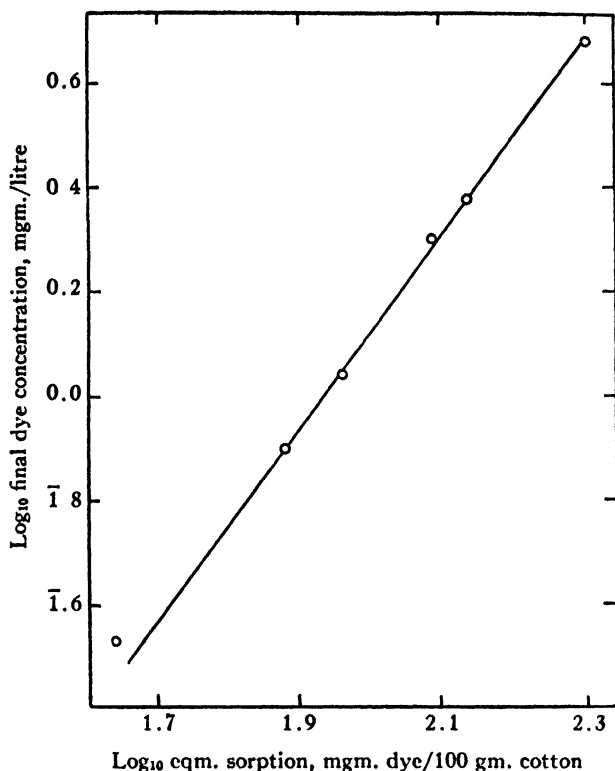


FIG. 3. Relation of equilibrium sorption at 90° C. to equilibrium concentration of dye in the bath. (Data obtained from curves of Fig. 2.)

Presence of Anionic Surface-active Agents

In Table I there are given the composition of the anionic surface-active agents tested, the percentage of surface-active material stated by the supplier to be present in each case, and the designations used later in the paper in referring to the agents.

Dyeing experiments were carried out at a temperature of 60° C., using an initial dye concentration of 26.7 mgm. per litre, with an anionic surface-active agent present in the dye-bath liquor at a concentration of 0.200 gm. per litre (1%, based on the weight of cotton yarn). In most of these experiments the dye sorption was measured over only the first two hours of the dyeing process.

The effect on the rate of dyeing, in the early stages of the dyeing operation, of the presence of surface-active agents is shown in Fig. 7. The heavy line in this figure is for a dyeing at 60° C. under similar conditions but in the absence of a surface-active agent, and the dotted line is for a comparable dyeing at

90° C. in the absence of surface-active agent. In order to eliminate crowding on the graph, the data for certain of the agents have not been plotted. The

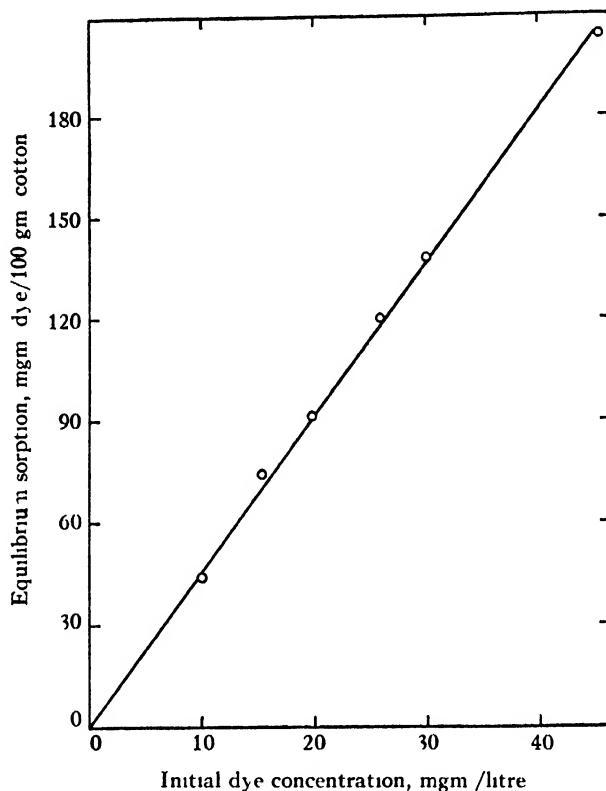


FIG 4 Relation of equilibrium sorption at 90° C to initial concentration of dye in the bath (Data obtained from curves of Fig 2)

TABLE I
ANIONIC SURFACE ACTIVE AGENTS USED

Nature of agent	Active material, %	Designation
Monobutyl phenyl phenol sodium monosulphonate	100	A
Dibutyl phenyl phenol sodium disulphonate	100	B
Decylbenzene sodium sulphonate	100	C
Dodecylbenzene sodium sulphonate	100	D
An alkyl (long chain) aryl sodium sulphonate	90*	E
An alkyl (long chain) aryl sodium sulphonate	40*	F
Disodium tri isobutyl succinate	100	G
Dioctyl sodium sulphosuccinate	100	H
Sodium salt of sulphated monoglycerides from coconut oil	98**	I
Sodium salt of sulphated monoglycerides from coconut oil	35**	J

* Remainder stated to be inorganic salts, chiefly sodium sulphate.

** Remainder stated to be sodium sulphate

curve for agent *C* fell almost on top of that for agent *E*, i.e., somewhat above that for the corresponding dodecyl compound, *D*. The curve for agent *B*

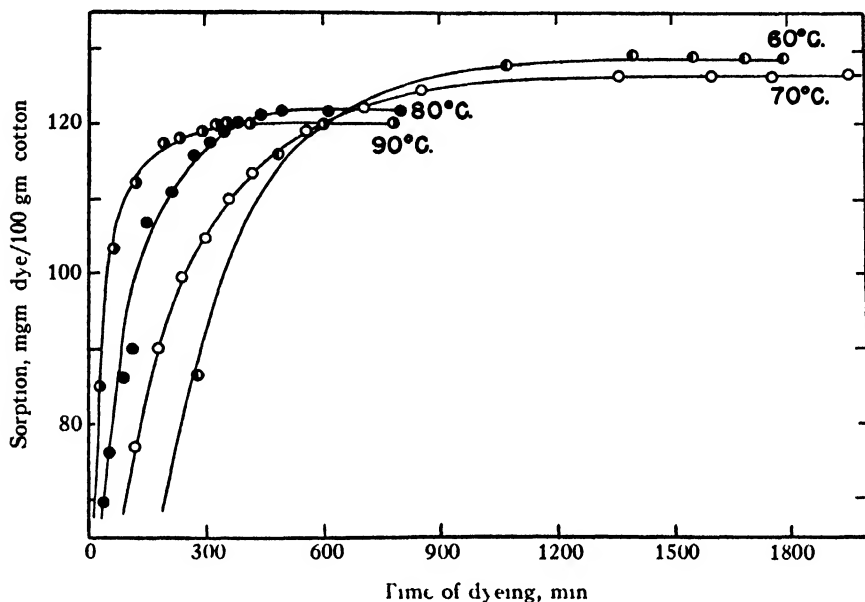


FIG. 5. Effect of temperature on the equilibrium sorption and the rate of its attainment.

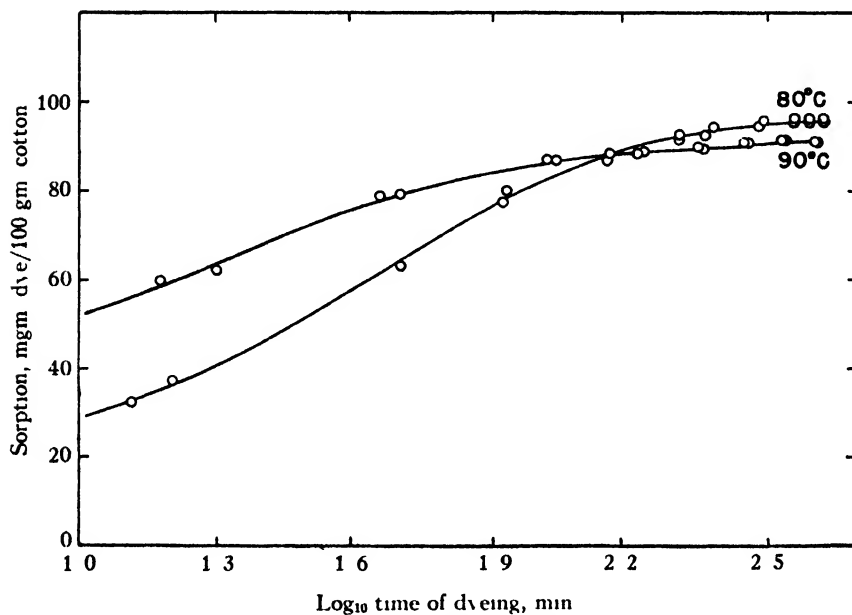


FIG. 6. Effect of temperature on the equilibrium sorption and the rate of its attainment.

fell below that of agent *D* and above that for the related monobutyl compound, *A*. The curve for agent *F* fell below that of the related agent *E*, close to that for *D*. Agent *G* was unique among the compounds investigated, in that it appeared to exert a slight rate-depressing effect.

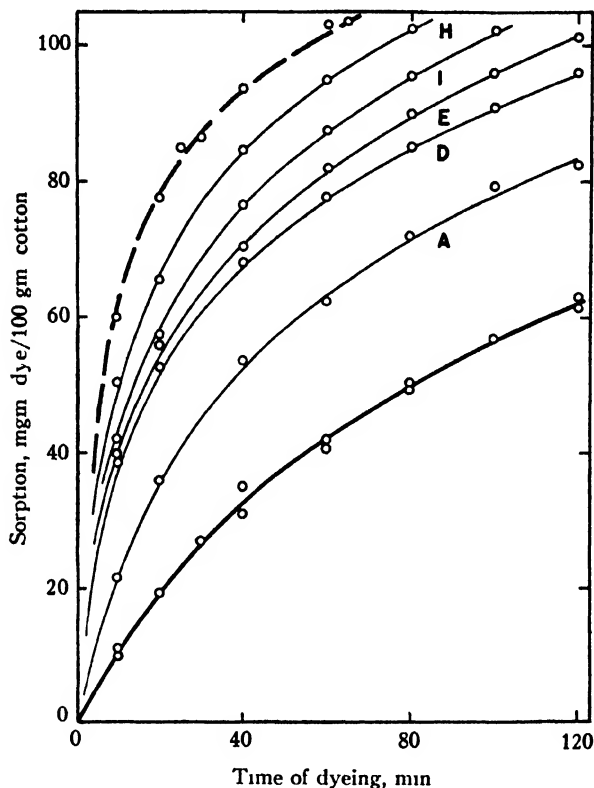


FIG. 7. Effect of commercial anionic surface-active agents on the rate of sorption at 60° C. (The heavy solid line is for a dyeing at 60° C., and the dotted line for one at 90° C., in the absence of surface-active agent.)

In Fig. 8 are plotted curves obtained from similar experiments, but in which methanol-soluble fractions of the surface-active agents were used, rather than the commercial products. The heavy and dotted lines in this graph have the same significance as in Fig. 7. Although the data are not plotted, it was found again that the curve for agent *C* lay slightly above that for agent *D*.

The effect of the purity of the agent is shown in Fig. 9. Curve 1 was obtained in the absence of surface-active agent, Curve 2 with the methanol-soluble fraction of agent *I*, Curve 3 with agent *J* and Curve 4 with agent *I* (all surface-active agents at a concentration of 0.200 gm per litre).

The activity of the surface-active agent as a function of its concentration is shown in Fig. 10. Curve 1 was obtained in the absence of surface-active agent, Curve 2 with surface-active agent *F* (commercial) at a concentration

of 0.040 gm. per litre, Curve 3 with the agent at a concentration of 0.100 gm. per litre, Curve 4 with a concentration of 0.200 gm. per litre, and Curve 5 with a concentration of 0.500 and 1.000 gm. per litre.

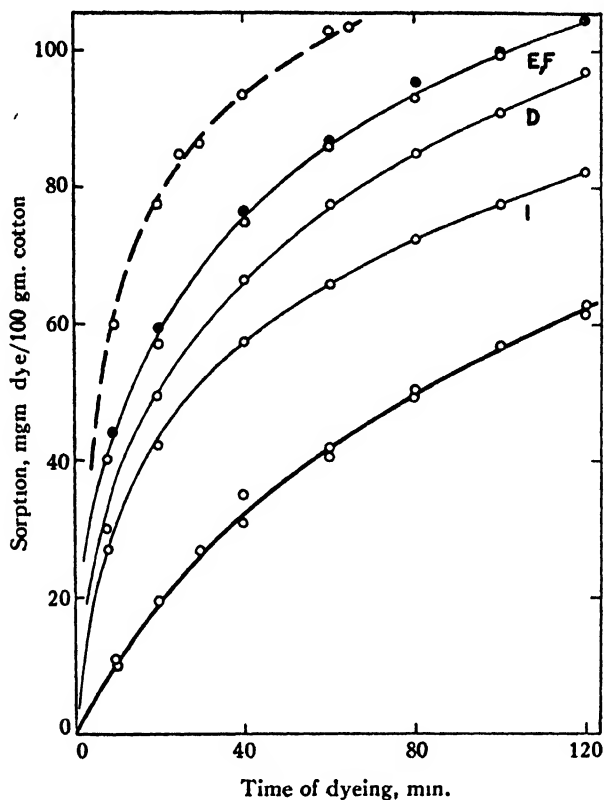


FIG. 8. Effect of purified anionic surface-active agent on the rate of sorption at 60° C.

Similar results were obtained using the methanol-soluble fraction of agent *I*; here again, the points for a concentration of 0.500 and 1.000 gm. per litre lay on the same curve.

The effect of a surface-active agent on the extent of dye sorption at equilibrium, as well as on the rate of sorption, is clear from Fig. 11, in which Curve 1 relates to a dyeing at 70° C. with an initial dye concentration of 26.7 mgm. per litre but with no surface-active agent present, and Curve 2 is for a dyeing under similar conditions but with the methanol-soluble fraction of agent *E* present in the dye-bath liquor at a concentration of 0.200 gm. per litre.

A similar pair of curves was obtained under the same conditions except for the use of a temperature of 60° C. At this temperature the effect of the surface-active agent on the equilibrium sorption (and on the rate of sorption) was somewhat more pronounced than at 70° C.; on the other hand, similar experiments at a temperature of 80° and 90° C. showed that the effect of the

surface-active agent on the equilibrium sorption was not detectable at these temperatures (although the effect on the rate was still observable at 80° C.).

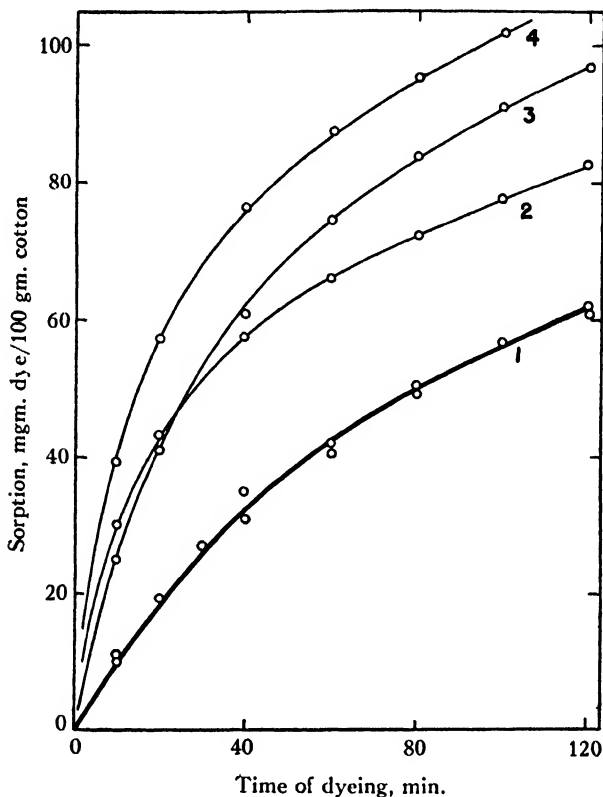


FIG. 9. Effect of purity of a surface-active agent on its activity at 60° C.

Discussion

It is evident from Figs. 2, 5, and 11 that the sorption of dye is relatively rapid during the early stages of the dyeing operation, but that the rate of dyeing decreases steadily as saturation, or the equilibrium sorption value, is approached. These dye-sorption-time curves are similar in shape to those that have been obtained during the study of the dyeing of viscose sheet with direct dyes (e.g., 9, 23, 24). The shape of the sorption-time curve is not, *per se*, evidence that any particular process, either chemical or physical, is the rate-controlling factor in the dyeing process.

Effect of Dye Concentration

From Fig. 2 it is clear that the time required to attain equilibrium conditions at 90° C. increases markedly as the initial concentration of dye in the bath is increased (concomitantly, the final or equilibrium concentration of dye in the bath is increased). The rate of dyeing (in terms of milligrams of dye sorbed

per 100 gm. of cotton in unit time), however, increases with increasing concentration of dye. Neale has found that the apparent diffusion coefficient of a direct dye into viscose sheet (determined under conditions of a very low

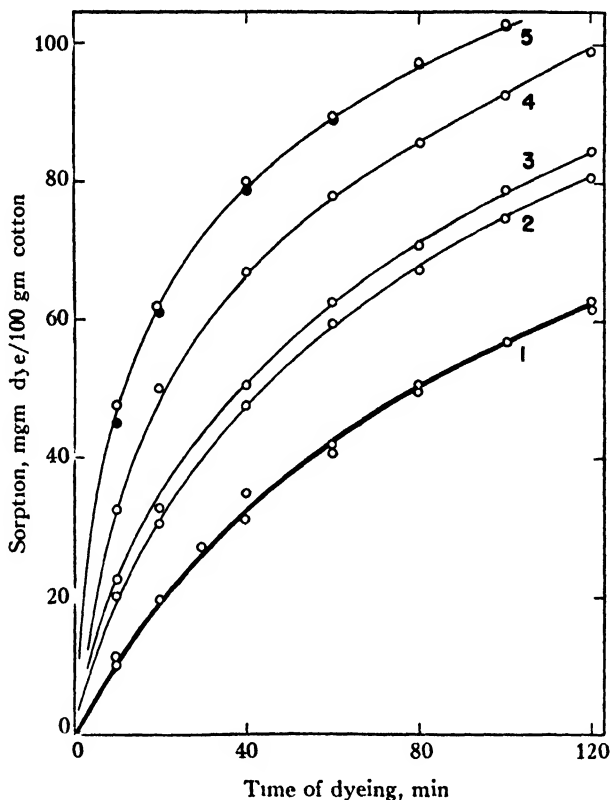


FIG. 10 Effect of concentration of a surface-active agent on its activity at 60° C.

degree of dye-bath exhaustion at equilibrium, rather than a very high degree as in the present experiments) increased as the concentration of dye in the bath increased (21), and that the diffusion coefficient of the dye increased with the concentration of absorbed dye (7).

An increase in the equilibrium sorption of direct dyes with increasing concentration of dye in the bath has been observed in the dyeing of viscose sheet (9, 24) and bleached cotton cloth (10), and the relation between sorption on viscose sheet and dye concentration found (7, 23, 41) to be expressible by a Freundlich equation.* That this equation represents the relation existing between the equilibrium sorption of Calcodur Blue 4GL on natural cotton

* $a = Kc^n$, where 'a' = amount of solute sorbed by a given mass of sorbent at equilibrium, 'c' = equilibrium concentration of solute in the solution, and 'K' and 'n' are constants. The equation is that of a 'generalized' parabola, $\log 'a'$ varies linearly with $\log 'c'$ (e.g., Fig. 3). McBain has noted (19, p. 5) that it is very incorrect to attribute this equation to Freundlich, as is commonly done.

yarn and the final or equilibrium concentration of dye in the solution is clear from Fig. 3. The applicability of this equation does not imply any particular mechanism for the dyeing process; the 'Freundlich' equation is an empirical one—as has been pointed out (29, p. 8), it "is a mathematical expression which will closely represent any chemical or physical phenomenon which proceeds at a diminishing ratio."*

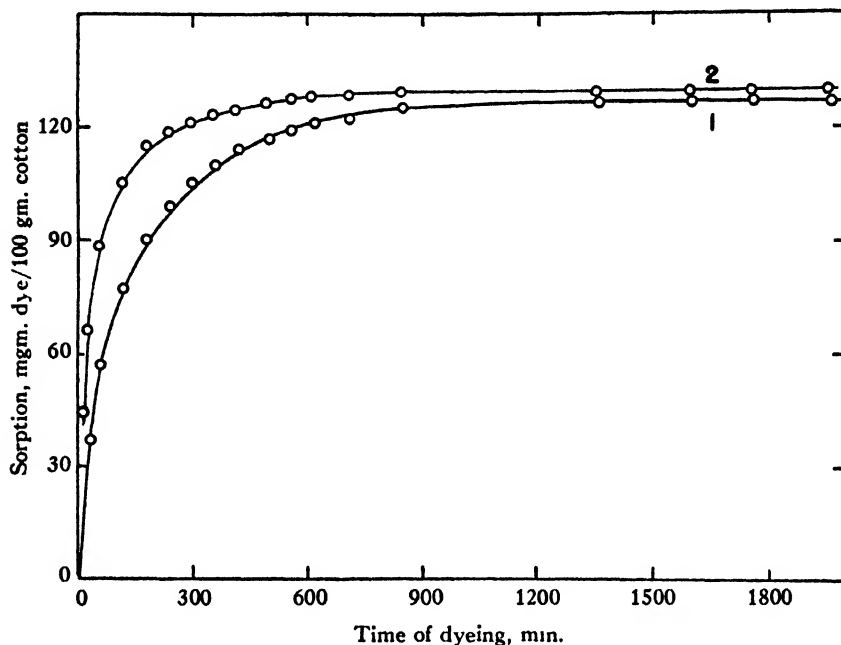


FIG. 11. Effect of surface-active agent on the equilibrium sorption at 70° C.

Fig. 4 shows that the relation between the equilibrium sorption value and the initial, rather than final, dye-bath concentration is a linear one, at least over the range of dye concentration studied. Because the ratio of the mass of dye liquor to that of cotton yarn was maintained constant, this linearity implies that the fraction of the dye initially present that is sorbed at equilibrium (or the degree of dye-bath exhaustion at equilibrium) is, in the present experiments, essentially independent of the initial dye concentration. This phenomenon, to which attention does not appear to have been drawn in the literature of the dyeing of cellulosic materials with direct dyes, would very likely be observable only under conditions leading to relatively high dye-bath exhaustions; in the experiments under consideration the exhaustions were all close to 92%. In experiments on the dyeing of cuprammonium yarn with direct dyes it has been shown (1) that the exhaustion of the dye-bath may

* It should be mentioned that a recently proposed theory of dyeing predicts (41) that the sorption isotherms, at constant salt concentration, will take the form of the 'Freundlich' equation.

decrease from close to 100 to below 20% when the initial dye-bath concentration is increased approximately 100-fold. It should be mentioned that in relatively recent work on the dyeing of viscose sheet with direct dyes (e.g., that by Neale and his co-workers to which reference has and will be made) the conditions of dyeing were so chosen that the decrease in the concentration of dye in the bath during dyeing was very slight (usually less than 2%). Such a technique facilitates theoretical interpretation, but it is, of course, far removed from commercial practice.

Effect of Temperature

It has been shown (6, 23) that when viscose sheet is dyed with direct dyes an increase in the temperature decreases the equilibrium sorption of dye but increases the rate of attainment of equilibrium; these effects of temperature are, of course, common to many types of sorption phenomena. That they are evidenced in the direct dyeing of natural cotton yarn is shown in Figs. 5 and 6. The sorption-time curves plotted with a logarithmic time scale (Fig. 6) are similar in shape to those that have been obtained for viscose rayon yarn (1, 2) and for cotton hairs (2). It should be noted that it has been found (6) that the equilibrium sorption of certain direct dyes on bleached cotton cloth is decreased by an increase in temperature; but a case has been reported (12) in which equilibrium dye sorption (on absorbent surgical cotton) increased with an increase in temperature.

A decrease in the value of the equilibrium sorption of Calcodur Blue 4GL on natural cotton with increasing temperature means that the dyeing process is an exothermic one. The heat of dyeing of cellulosic material with direct dyes would appear to have been assessed for only two dyes (16, p. 274; 41), and these determinations were made from the temperature coefficient of the equilibrium sorption on viscose sheet. It is planned to undertake in this laboratory an investigation of the heats of dyeing of natural cotton yarn by a number of direct dyes.

Effect of Anionic Surface-active Agents

The rate of direct dyeing of mercerized cotton yarn at room temperature has been shown (4, 31) to be accelerated in the presence of certain anionic surface-active agents. On the other hand, the rate of dyeing of cotton pongee with a direct dye at 25° C. in the presence of a number of anionic surface-active agents has been shown (32) to be either increased or decreased (at least during the first hour of the dyeing process), depending on the concentration of sodium chloride present. From Figs. 7-11 it is evident that anionic surface-active agents of the sulphonate (either alkyl aryl or aliphatic) type and sulphated aliphatic esters accelerate the rate of sorption of Calcodur Blue 4GL on natural cotton yarn in the presence of sodium chloride (4.00 gm. per litre). The statement by Snell (34) that "an anion-active agent added to a bath containing acid dye, since each gives a large negatively charged ion, slows

down the sorption according to the amount of agent added" cannot, therefore, be accepted.

In some cases the rate-accelerating effect obtained at 60° C. due to the addition to the dye-bath of 0.2 gm. per litre of a surface-active agent is almost equivalent to that obtained by raising the temperature, in the absence of a surface-active agent, to 90° C. (e.g., see Fig. 7). All the surface-active agents showing a positive effect on the rate at 60° C. were more effective (at a concentration of 0.2 gm. per litre and, in at least one case, at a tenth of this concentration) than raising the temperature 10° C., in the absence of a surface-active agent.

A comparison of Figs. 7 and 8 shows that the methanol-soluble fraction of agent *D* has essentially the same activity as the commercial product, whereas the methanol-soluble fraction of agent *I* has a significantly reduced activity; this reduction in activity is shown more obviously in Fig. 9. The data plotted in Fig. 9 show that the effect of commercial surface-active agents on the rate of sorption is not due, or at least not largely due, to the presence of inorganic salts in the surface-active preparations because agent *I*, which is 98% 'active,' has a significantly greater effect than agent *J*, which is 35% 'active' (the remainder is stated to be sodium sulphate). The methanol-soluble fraction of agent *I* has a smaller effect on the rate than the unpurified agent *I* presumably because that fraction of the surface-active material present that is soluble in methanol is rather less 'active' than the fraction of surface-active material that is not extracted by methanol. The reduction in the rate-accelerating effect of a surface-active preparation due to the presence of inorganic salts is again evidenced in the data obtained with agents *F* and *E*. It has been noted that the rate-accelerating effect of agent *E* (90% 'active') is greater than that of agent *F* (40% 'active'), and consideration of Figs. 7 and 8 will show that the methanol-soluble fractions of these agents not only exhibit the same activity, but an activity greater than that of the commercial agent *E*.

The dependence of the rate-accelerating effect of a surface-active agent on its concentration is shown in Fig. 10. With this agent (*E*) the maximum rate effect, at least in so far as the early stages of the dyeing process are concerned, is achieved at a concentration of 0.5% or less (but greater than 0.2%); a similar result was found for the methanol-soluble fraction of a different agent (*I*), as noted earlier.

It is the authors' view that anionic surface-active agents exert their effect by virtue of an ability to promote wetting and swelling of the cotton fibres. These physical effects would result in an increased rate of sorption, as has been found in this work. Once the available cellulosic surface is saturated with surface-active agent (oriented with the polar or hydrophilic group facing the aqueous phase), an increase in the concentration of the latter would have

no effect; this optimal value for the concentration of surface-active agent has been observed (Fig. 10). In Fig. 11 it is shown that the presence of a surface-active agent may result in an increased value for the equilibrium sorption. This phenomenon, apparently not previously observed, is understandable, and to be expected, if an action of the surface-active agent is to increase the swelling of the fibre, thus increasing the capillary dimensions and/or making available minute channels and intermicellar spaces that would not have been available for dye penetration in the absence of the surface-active agent; the latter, in this sense, creates additional surface upon which dye may be sorbed.

It has been noted in the section dealing with experimental results that the activity of an anionic surface-active agent (purified agent *E*) decreases, both as regards effect on the equilibrium sorption and the rate of its attainment, as the temperature is increased. This effect may be due to a decrease, with increasing temperature, of the wetting ability of this surface-active agent. Although it is, perhaps, more common for the wetting power⁴ of an anionic surface-active agent to increase as the temperature is raised, the effect of temperature on this property is variable (3, 5); a case has been reported (3), for example, in which the wetting power (measured by the Draves test using a skein of gray cotton yarn) of an anionic surface-active agent goes through a maximum as the temperature is raised and then falls off rapidly at temperatures above 55° C.

It was noted earlier that spectrophotometric tests showed that the surface-active agents had no appreciable effect on the absorption spectrum of the dye in the presence of salt. If the effect of the surface-active agent did not arise, as suggested above, through an action on the fibre, but as a result of changing the degree of aggregation or association of the dye, then one would expect to find evidence of this in the spectrophotometric work[†], although this work was carried out at room temperature and the dyeings were at an elevated temperature (the aggregation of dyes decreases with increased temperature (2, 32, 35)). The effect of surface-active agents on particle size of dye micelles has been inferred from their effect on the aqueous diffusion coefficient of the dye (32, 35, but cf. 20). A few investigations of this nature have been carried out, and reviewed by Standing (35); some surface-active agents, such as certain cationic ones, very appreciably reduce the diffusion coefficient[‡] (and thus, presumably, greatly increase the aggregation of the dye micelles), whereas the anionic surface-active agents investigated affected the aqueous diffusion

* Snell has pointed out (34) that "the term 'wetting power' is variously used as meaning the process of wetting, the degree of wetting, the ease of wetting, or the speed of wetting." Both the Herbig and Draves tests primarily measure speed of wetting.

† β -naphthol, which is sometimes used as a levelling agent for direct dyes, has been found (28) to have no effect on the spectrum of Chlorazol Sky Blue FF, nor, at the concentration used, on its 'absorption' by bleached Egyptian cotton cloth. On the other hand, β -naphthol has been reported to increase the speed of dyeing (1, 2) and decrease the dye sorption (2) in some cases.

‡ Cationic surface-active agents are employed as levelling agents in the direct dyeing of cotton, and it has been suggested (33) that they function as such by their action as retardants for the dyeing rate.

of the dye only slightly. This constitutes further evidence that anionic surface-active agents exert their effect primarily not through an action on the dye, but as a result of one involving the fibre (32, 33, 39).

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RESIN-RUBBER FROM CANADIAN GROWN PLANTS

VI. PEBBLE-MILLING MILKWEED LEAVES IN CLOSED CIRCUIT¹

BY R. V. TOMKINS² AND N. H. GRACE³

Abstract

Conversion from batch to closed-circuit continuous operation increased the capacity of a pebble-mill from 5 to 14 5 lb. solids per hour when grinding digested milkweed leaves to pass 60 mesh for subsequent separation of resin-rubber by froth flotation. The rate of grind is a decreasing function with time in the mill; this indicates use of large mill discharge and recycle rates for high capacity.

Introduction

Attention has recently been given to milkweed leaves as a source of a resin-rubber gum for blending with GR-S, and a pilot plant process consisting of digestion, pebble-milling, froth flotation, and recovery from the concentrate has been described (1). The pebble-milling operation was performed batchwise and its conversion to closed-circuit continuous milling appeared desirable since the limiting factor of the batch process was the capacity of the pebble-mill. Continuous operation should increase the capacity of the mill and reduce labour requirements, which were the major cost factors in batch milling.

Also, continuous closed-circuit milling has the advantage of removal of the ground product as soon as, or shortly after, it has reached the desired size. This eliminates overgrinding and, in this instance, minimizes the tendency of the cellulosic material to gelatinize or swell as a result of prolonged milling. The capacity of the mill when used batchwise was 15 to 20 lb. of solids for each 3 5 hr. milling period, or approximately 5 lb. of solids per hour. If even this rate could be achieved by continuous operation, the time and labour necessary for loading, draining, and screening would be eliminated.

This investigation included determination of a typical batch grinding rate, preliminary continuous runs to check operation of equipment under various feed conditions, and final runs for determination of capacity obtainable with the auxiliary equipment available. As the product was not in demand, and the supply of milkweed leaves limited, the aim of this work was the determination of the practicability of such continuous milling rather than a detailed study of the various operations. However, the information obtained may be of use for the development of similar milling processes, as literature on this type of operation is rather meagre.

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Equipment

The units comprising the milling circuit are shown in their relative positions in Fig. 1. The digested milkweed leaves (1) were fed by a screw conveyor through a hollow trunnion of the pebble-mill and discharged through the other trunnion to the vibrating screen. The oversize flowed into the boot of the bucket elevator, which returned it to the feed end of the mill for further grinding. The undersize was pumped to a calibrated storage box for subsequent froth flotation.

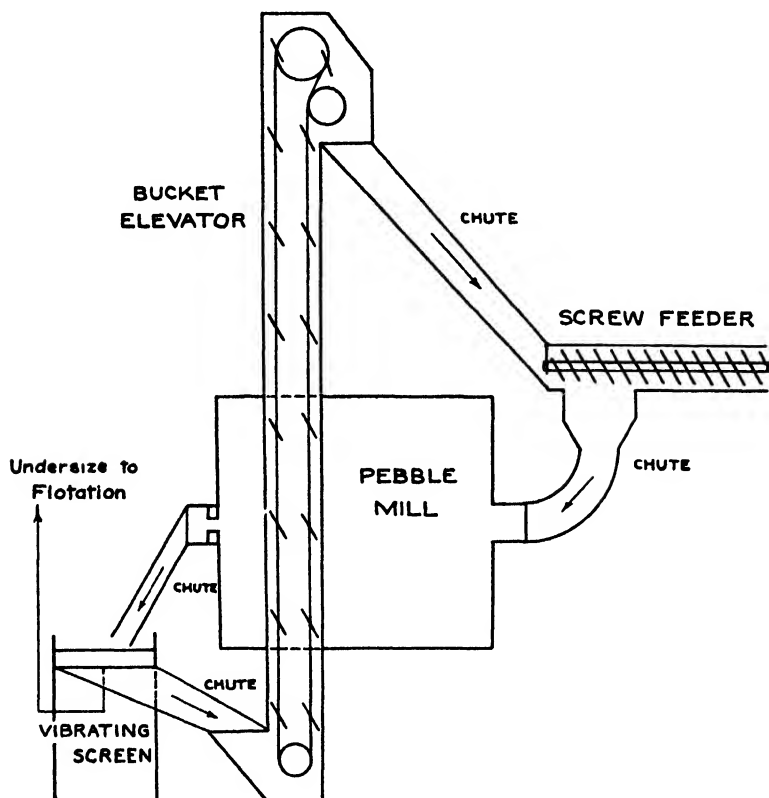


FIG. 1. Milling circuit for continuous grinding of digested milkweed leaves.

Feeder

An open spiral conveyor with a 4 in. diameter screw rotating at 1.5 r.p.m. fed the digested leaves to the pebble-mill. The feeder served to smooth the flow, rather than to meter the feed, which was weighed and brought to the desired consistency before being placed in the conveyor.

Pebble Mill

A Straub mill, 36 in. diameter by 36 in. long, gross capacity 130 gal., lined with porcelain brick was used to comminute the digested leaves. Flint pebbles (average diameter, 2.3 in.) occupied about 25% of the gross volume.

The mill was rotated at 32 r.p.m. (80% of theoretical critical speed). The feed was admitted through a metal chute attached to the bearing housing to a hollow trunnion having a 6 in. diameter opening. The discharge end was fitted with a wooden plug containing a hole 2 in. in diameter, small enough to prevent the pebbles from leaving the mill.

Vibrating Screen

The ground material was carried by an open chute from the mill to a Dillon vibrating screen. The screen used was 60 mesh stainless steel, 18 by 48 in., oscillating about 30 times per sec. and sloped 5° to the horizontal.

Bucket Elevator

The elevator carried 16 buckets of 28 cu. in. capacity, travelling 75 ft. per min., thus having a maximum capacity of 2700 lb. per hr. of the oversize material. The lift was about 10 ft., allowing the material to flow by gravity back to the mill.

Experimental Procedure and Results

The milkweed leaves used throughout this investigation were prepared by digestion and washing as previously described for batch milling (1).

Moisture determinations were made by drying at 105° C. for 48 hr. Resin-rubber contents were obtained by successive 24-hr. acetone and benzene extractions (2).

Batch Milling

At various times during a batch run 20-lb. samples of slurry were removed and passed over the vibrating screen. The oversize was collected and weighed, and solid content of the mill slurry and the oversize determined. In addition a 20 lb. sample of unmilled material was screened. The solid content of the mill slurry was 5.4%. The points on the curve in Fig. 2 were obtained by calculation from these data.

Although the conditions in the mill during batch operation are somewhat different from those during continuous operation, certain general conclusions may be applied. The instantaneous rate of grind is given by the slope of this curve (Fig. 2) at any point, and, assuming the form of the curve to be typical, the rate is a decreasing function with time, being greatly reduced during the first hour. Therefore it is desirable to have a high discharge rate from the continuous mill, corresponding to a short period of milling per pass, in order to obtain the advantage of the higher rates of grind.

Continuous Milling

The capacity of the system is limited by the rate of grind attained in the mill, but it is possible that the recycle equipment may not be large enough to allow the maximum capacity to be attained.

The consistency of the feed is limited by the minimum solid content desired for flotation (2%) and the solid content of the cooked washed leaves (5 to 7%). Below 2%, thickening would be necessary for satisfactory flotation.

The following balances are applicable if the system is in equilibrium:

Around the whole circuit:

$$F = U \quad (1)$$

$$fF = uU \quad (2)$$

Around the feeder:

$$F + R = M \quad (3)$$

$$fF + rR = mM \quad (4)$$

Around the screen:

$$U + R = M \quad (5)$$

$$uU + rR = mM. \quad (6)$$

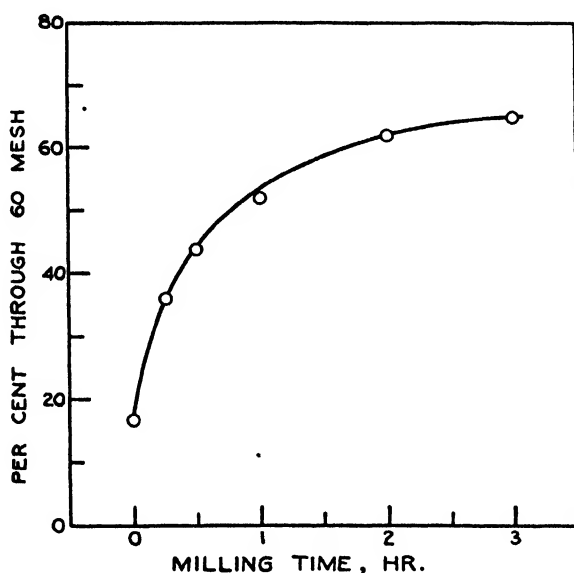


FIG. 2. Grinding rate on batch milling.

The fractional solid contents of the feed, undersize, recycle, and mill discharge are represented by f , u , r , m , respectively. The corresponding capitals indicate gross flow rates in pounds per hour.

The test for equilibrium is that Equations (1) and (2) must be satisfied. However, as slight losses are inevitable in the system the criterion that

was selected.

$$f = u \quad (7)$$

The quantities U and F are easily measured, but as R and M are somewhat unsteady owing to slight variations in feed, only approximate values could be obtained during the runs. From (3) and (4) the following equations were obtained:

$$R = \frac{F(m-f)}{(r-m)} \quad (8)$$

$$M = \frac{F(r-f)}{(r-m)}. \quad (9)$$

The right-hand values could all be measured, but, as the bracketed factors have very small values, the error in the calculated values may be large. The figures given further on were a compromise between observed and calculated values.

An estimate of the average rate of grind can be made from the following considerations. The fraction of solids going through 60 mesh per pass through the mill is given by

$$K = \frac{uU}{mM} = \frac{fF}{mM} \quad (10)$$

The mill load is about 350 lb. of slurry. Then the average time (hours) in the mill per pass

$$t = \frac{350}{M} \quad (11)$$

The average rate of grind, b , is obtained by dividing Equation (10) by Equation (11)

$$b = \frac{K}{t} = \frac{fF \times M}{mM \times 350} = \frac{fF}{350m} \quad (12)$$

This value gives a rate of grind for comparison with the slope of the curve in Fig. 2.

The procedure for making continuous runs follows. The mill was charged at the desired solid content and run without feed for two hours. Feeding was then begun at the desired rate by placing weighed amounts of leaves of the required solid content in the feeder at 10-min. intervals. Preliminary runs were designed to check equipment operation under various feed conditions. Samples were taken of the undersize four hours after feed started and the solid contents determined to discover whether equilibrium had been established.

Run 1. Feed: 250 lb. per hr. at 2% (5 lb. solids per hr.). Undersize: 1 95% solids, equipment operation satisfactory. Equilibrium established.

Run 2. Feed: 125 lb. per hr. at 4% (5 lb. solids per hr.). Undersize: 3 6% solids, equipment operation satisfactory. Equilibrium not established.

Run 3. Feed: 400 lb per hr. at 2% (8 lb. solids per hr.). Undersize: 1 9% solids, equipment operation satisfactory. Equilibrium closely approached.

Run 4. Feed: 200 lb. per hr. at 4% (8 lb. solids per hr.). Undersize: 3 5% solids, the screen was somewhat overloaded and mill discharge plugged at intervals by underground material. Equilibrium not established.

This led to the selection of about 2% as the solid content of the feed for final runs. This also gives larger total feed rate (pounds per hour) for a given solid feed rate (pounds of solids per hour), thus possibly resulting in higher mill discharge rates and higher rates of grind. During the final runs,

samples were taken hourly. The feeds contained 2.3% solids. The results from runs at 5.75, 11.5, and 14.5 lb. solids per hr. are given in Table I. In all these millings, equilibrium was established, the minor fluctuations being due to variation in the feed. A run at 17.5 lb. solids per hr. was unsuccessful, as the bucket elevator could not handle the recycle.

TABLE I
CONTINUOUS MILLING RUNS

Feed	Hours run*	Undersize, % solids	Recycle		Mill discharge	
			Lb./hr. (estimated)†	% solids	Lb./hr. (estimated)†	% solids
5.75 lb. solids/hr. (250 lb./hr. at 2.3% solids)	2	1.7		5.3		2.8
	3	1.9		4.5		2.8
	4	2.3		4.6		3.0
	5	2.2	200	4.4	450	3.1
11.5 lb. solids/hr. (500 lb./hr. at 2.3% solids)	2	2.5		4.4		3.8
	3	2.4		4.4		3.5
	4	2.3		4.3		3.9
	5	2.4	1000	4.3	1500	3.9
14.5 lb. solids/hr. (625 lb./hr. at 2.3% solids)	2	2.2		4.0		3.1
	3	2.1		3.9		3.4
	4	2.3		3.7		3.2
	5	2.2	1800	3.8	2425	3.6

* From beginning of continuous feed.

† See text.

The slurries obtained from these runs were subsequently passed through the froth flotation cell and this operation proceeded as efficiently as when batch-milled material was used. Analyses of the slurries for resin-rubber were compared with the resin-rubber content of the unmilled leaves and were found to be substantially the same; this indicated no accumulation of resin-rubber in the mill.

Table II shows the average values for rates of grind. Although these values are only approximate, they show that, as predicted, the rates of grind increase very appreciably as the time of passage through the mill decreases.

The vibrating screen and elevator do not have enough capacity to match the mill. Higher grinding rates could be obtained with larger recycle equipment, the maximum being attained only when the rate of discharge of the mill has reached its limit, as determined by the size of the discharge opening and viscosity of the slurry. However, the recycle rate increases greatly as feed rate is increased and the necessary equipment would probably be so large as to overshadow any advantage of high milling capacities.

Here, the batch rate has been nearly tripled by continuous operation, and the material appears well suited to closed-circuit milling.

TABLE II
AVERAGE RATES OF GRIND ATTAINED

Feed, lb. solids/hr. (fF)	Mill discharge		k , fraction through 60 mesh per pass	t , hours per pass	$b = k/t$ fraction through 60 mesh per hour
	Solids, % (at 5 hr.)	Lb./hr. (at 5 hr.)			
5 75	3 1	450	0 42	0 78	0 53
11 5	3 9	1500	0 20	0 23	0 86
14 5	3 6	2425	0 17	0.15	1.15

Acknowledgment

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AN ACCURATE MOUNT FOR TRI-METROGON PHOTOGRAPHY¹

By R. A. NODWELL² AND R. C. BURSTOW³

Abstract

A tri-camera mount that fits into the nose of a Mitchell B-25 aircraft and that accurately maintains the interaxial relation between the cameras is described. The alterations and adjustments of the Fairchild K-17 cameras to give the necessary accuracy and interchangeability are also described. Apparent inaccuracies in the mount are discussed, and it is concluded that the main source of error is the instability of the photographic film, which may lead to angular errors of 12 min.

Introduction

In the Canadian method of tri-metrogon tilt analysis outlined by Carroll (2) great saving in time and labour can be effected if the cameras are held rigidly in such a way that the following relations are true:—

- (a) The optical axes of the three cameras are parallel to a plane, usually vertical;
- (b) The line joining the transverse fiducial marks in each camera are parallel to the same plane;
- (c) The interlocking angles between the optical axes of the vertical and oblique cameras are reliably known.

The design and construction of a rigid tri-camera mount that would maintain these relations was undertaken by the Optics Section of the National Research Laboratories, Ottawa, at the request of the Canadian Photographic Research Committee. The mount was to be installed in a Mitchell B-25 aircraft and carry three Fairchild K-17 cameras with Bausch & Lomb 6 in. metrogon lenses. It was specified that the plane defined in each of the cameras by the principal axis and the line joining the fiducial marks transverse to the line of flight be parallel to similar planes in the other two cameras within two minutes of arc. The angles between the principal axis of the vertical camera and those of the oblique cameras were to be approximately 60° with the angles known within three minutes of arc. It was also requested that all cameras be made interchangeable, if practicable, to reduce the seriousness of a breakdown in any one of the cameras by making possible the immediate substitution of a spare.

Alterations to the Cameras

In order to eliminate any possibility of inaccuracy because of movement between the magazine, which carries the fiducial marks, and the camera body the fiducial marks were mounted on the camera body. This is accomplished

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by mounting a plate permanently on the back of the camera (see Fig. 1). This plate duplicates the original camera back except for a raised shoulder $\frac{3}{8}$ in. high, which projects inside the picture frame of the magazine so that the suction back presses on the shoulder when the magazine is in the cocked position. The fiducial marks are mounted on this shoulder. The shoulder reduces the picture size to $8\frac{3}{4}$ by $8\frac{1}{2}$ in., but it was felt by those responsible for the photogrammetry that this disadvantage was compensated for by the added certainty in determining the position of the principal point.

To simplify the procedure in calibrating survey cameras it is desirable to open and close the shutter without disassembling the camera. Unfortunately, no provision is made for this in the K-17 camera. This deficiency is overcome by cutting a slot in the end of the wind gear shaft. The shutter may then be opened or closed by removing the taper pin that locks the wind gear to the shaft and turning the shaft by means of a screwdriver.

Since a dark slide cannot be inserted when the magazine is mounted on the camera, a method was devised to make the magazine light tight while it is being removed from the camera. This is done by replacing the magazine cover locking knob by one of larger diameter and tapping this new knob to take a threaded shaft. The latter shaft, when screwed down, pushes the suction back on to the lip of the magazine. Tests have shown that this makes the magazine light tight. Since the action lifts the magazine slightly the release of the magazine catches must be the first operation performed when removing the magazines. A handle was put on each end of the magazine to facilitate handling.

The Mount

The Mount

The camera mount is a welded joint construction of 1 in. diameter steel tubing reinforced with No. 14 gauge sheet metal. To suit the aircraft the width of the mount had to be kept to a minimum and hence the oblique cameras are located above the vertical one. A completed mount with the cameras is shown in Fig. 2. The mount is insulated from the vibration of the aircraft according to the general method proposed by Reid (4). It is supported on 300 sq. in. of 2 in. thick sponge rubber (medium Dunlopillo) at the plane containing the centre of gravity of the whole assembly. In operation the loading of the rubber is approximately 1 lb. per sq. in.

The details of the method of holding the cameras in the mount are shown in Fig. 3, which is a view from inside the mount. The trunnion *A* has been removed from the camera (not shown) for purposes of illustration. Rotation of the trunnion *A* about the pin *B* permits adjustment of the principal axes of the cameras into parallel planes. The yoke *C* is reamed to fit accurately the shoulder of the screw *D*, which also fits a counterbored hole in the knee *E*. Rotation of the yoke about the screw *D* permits adjustment of the fiducial marks into the correct planes. One of the yokes *C* for each camera has a slot, instead of a reamed hole, which accurately fits screw *D* and allows some motion in the fore and aft direction to accommodate variations in

PLATE I

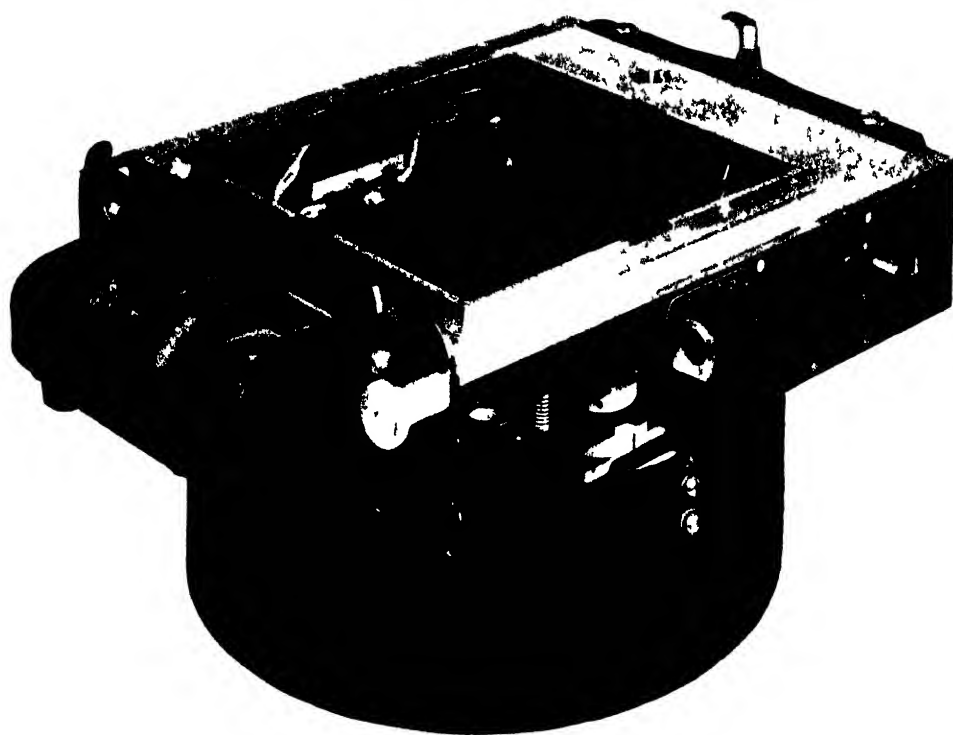


FIG. 1 A modified K 17 camera

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PLATE II

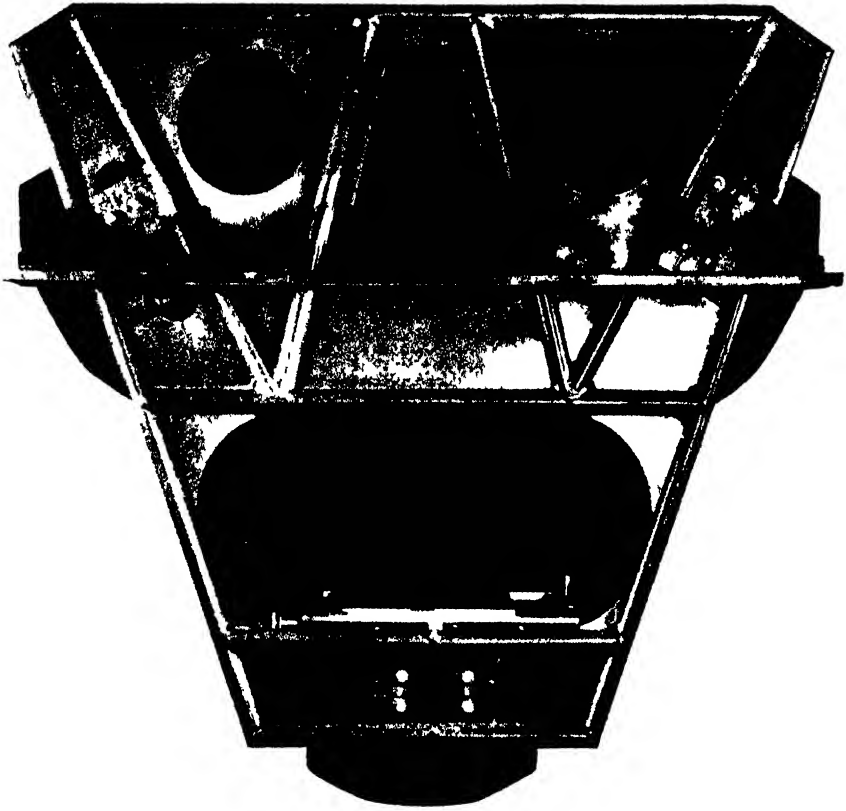


FIG. 2 The mount

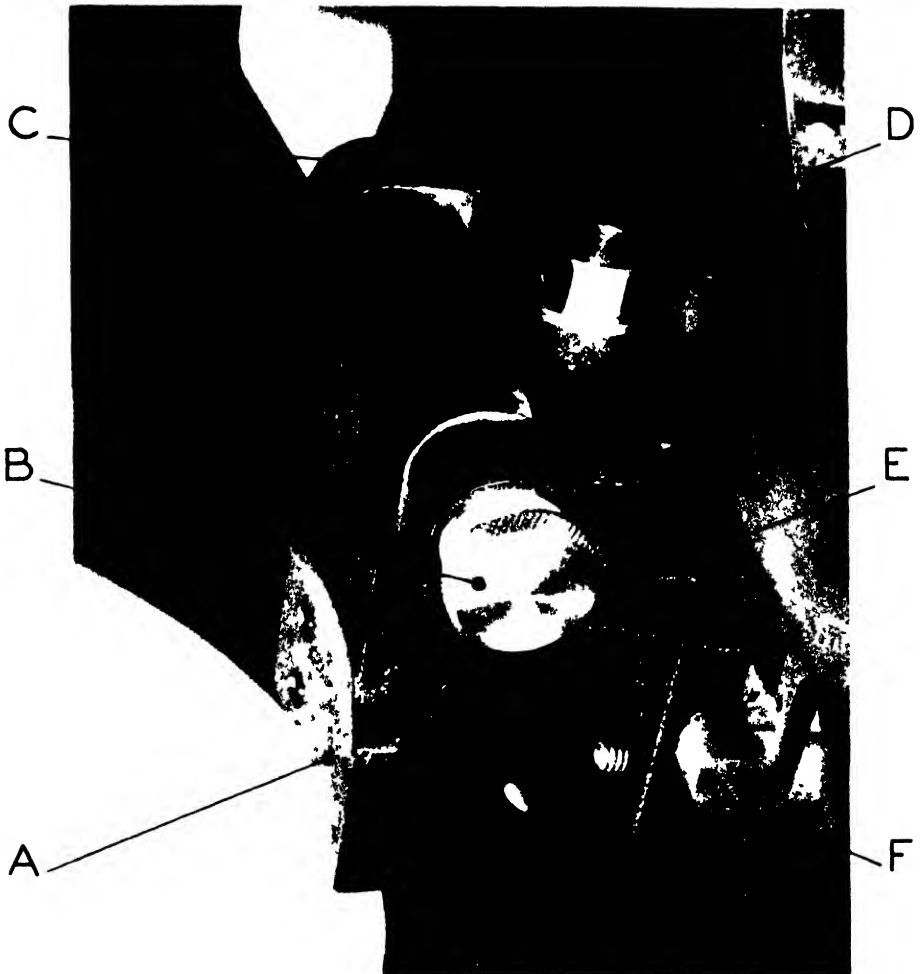


FIG. 3 The method of positioning the camera in the mount

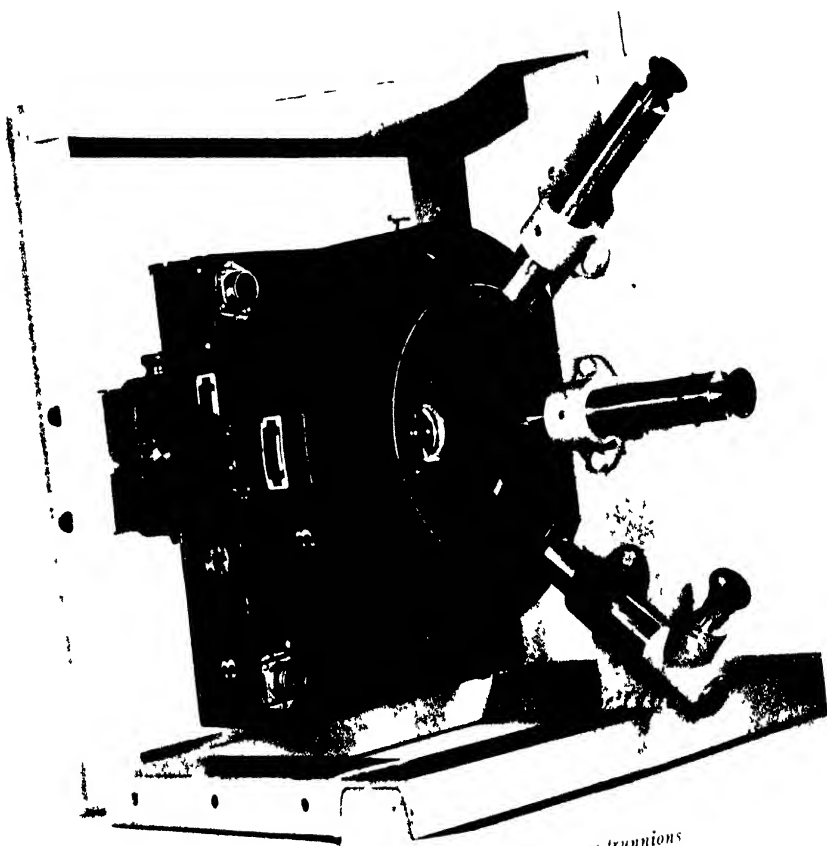
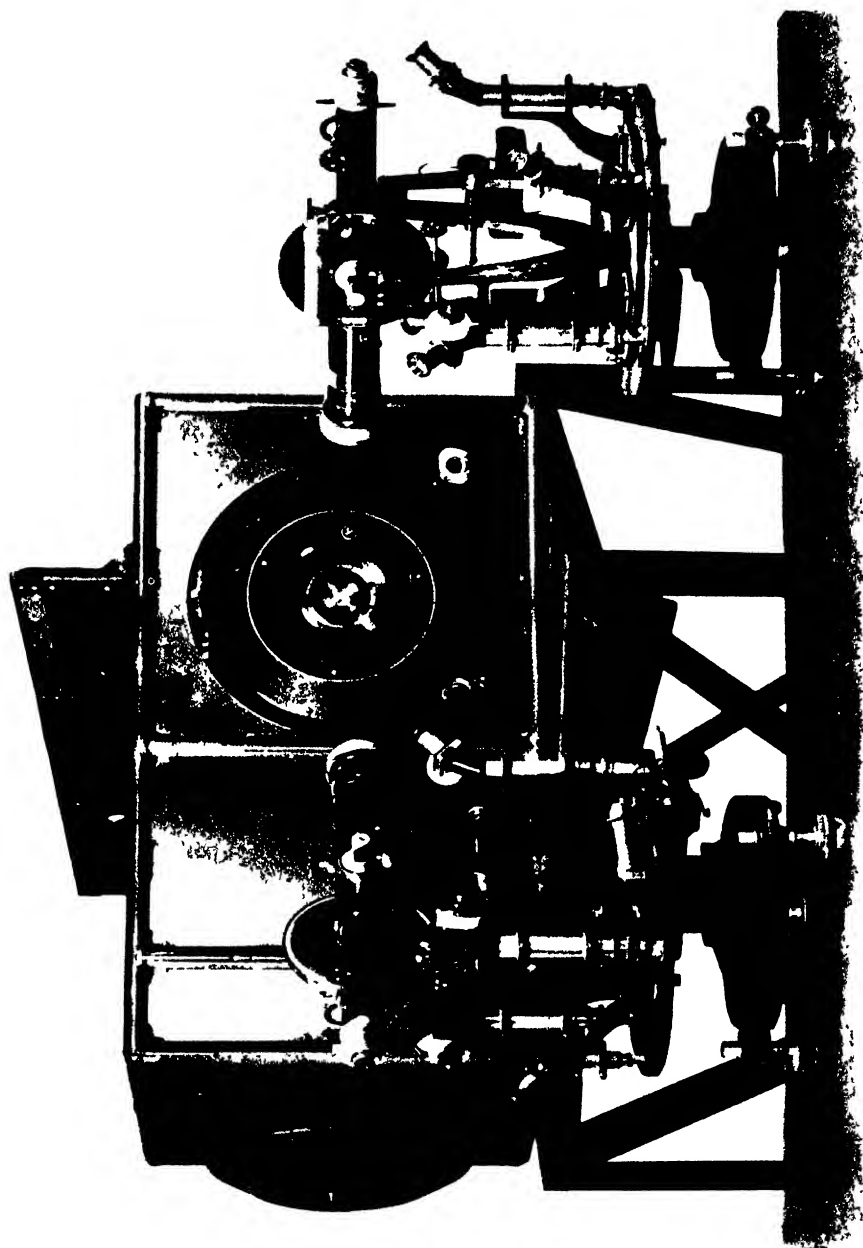


FIG. 4 The optical jig for locating the camera trunnions

PLATE V



camera diameters. The knee *E* can be moved in two directions by means of opposing screws *F*. All surfaces are machined to tolerances necessary for snug fits and interchangeability.

Method of Adjustment

The location of the principal point and measurement of principal distance of each camera is done by the method outlined by Field (3).

The location of the trunnions of each of the cameras relative to the fiducial marks is accurately established by means of the optical jig shown in Fig. 4. A camera, with its principal point defined by a graticule, is mounted in the rigidly located yokes, and the three telescopes are lined up on the principal point and the two transverse fiducial marks. The first camera is removed, a second camera inserted and shifted on its trunnions until the fiducial marks and principal point line up with the telescopes. When this condition is obtained the trunnions are locked with taper pins. All the cameras are adjusted in this manner and hence are interchangeable.

The method of adjusting the cameras in the mount is as follows (see Fig 5):—

The mount is placed on its side on top of a welded iron table. The three cameras, each with a graticule defining its principal point, are mounted in position. Two transits are set up to look at the transverse fiducial marks of one camera and levelled. The camera is adjusted by means of the opposing screws (*F*, Fig. 3) until the fiducial marks coincide with the cross hairs of the telescopes. The transits are then moved to the next camera and the process repeated and so on until the plane defined by the transverse fiducial marks of each of the cameras is horizontal. When these planes have thus been made parallel the trunnion knees (*E*, Fig. 3) are locked with taper pins. The angles between the principal axis of the vertical cameras and those of the oblique cameras are measured with the transits.

Performance of the Mount

Three mounts were constructed and installed in aircraft and were used in the summer survey of 1945. The interlocking angles found from the photographs did not agree with the calibrated angles. Typical results from test photographs are shown in the graph of Fig. 6, in which the abscissa is the picture number taken at 5-sec. intervals and the ordinate is the indicated angle between the vertical and the oblique camera. The calibrated angle for the left oblique was $60^{\circ} 37'$ and for the right oblique, $60^{\circ} 39'$. It will be noted that errors up to ± 2 min. are found in the angle.

The sources of error that might account for this discrepancy and that have therefore been investigated in detail are:—

1. Mechanical instability of the mount,
2. Thermal instability of the mount,
3. Lack of synchronization of the shutters,
4. Instability of the film base.

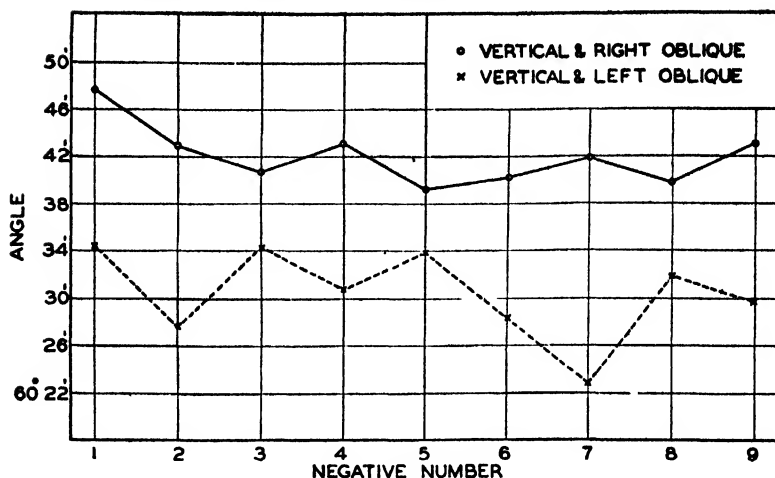


FIG 6 Variation in the angle calculated from photographs taken at five-second intervals. The calibrated angle for the left oblique was $60^{\circ} 37'$, and for the right oblique was $60^{\circ} 39'$.

Mechanical and Thermal Stability

The mechanical and thermal stabilities of the mount were checked in the laboratory. The design calculations, which had indicated that the mount would be adequately rigid, were corroborated. No change in calibration was noted for a temperature change of 60°F .

Shutter Synchronization

A test of the synchronization of the shutters showed that the maximum time lag between any two of the cameras tested was 10 milliseconds. Under the most adverse conditions of aircraft roll this could lead to an error of three minutes.

Instability of the Film Base

The method of calculating the interlocking angle from the photographs will be made clear by a reference to Fig 7, in which O is the perspective centre and OA and OB the optical axis of the vertical and oblique cameras, respectively. The lines AM_v and BM_o represent the traces of the focal planes, A and B the principal points, and M_v and M_o the fiducial marks of their respective cameras. Conjugate image points lying on or near the line joining the transverse fiducial marks are represented as C_v and C_o . The interlocking angle may be calculated by the formula

$$\theta_v = \tan^{-1} \frac{AC_v}{OA}$$

$$\theta_o = \tan^{-1} \frac{BC_o}{OB}$$

$$\theta = \theta_v + \theta_o$$

These formulae would be accurate for measurements on plate but, normally, distances measured on film are inaccurate owing to change in dimension of

the film base following processing (1). To compensate for this factor the fiducial distance (the distance from fiducial mark to principal point) had been measured on photographic plate for each of the cameras. The distance from the fiducial mark to the image point (M_vC_v) was measured and subtracted

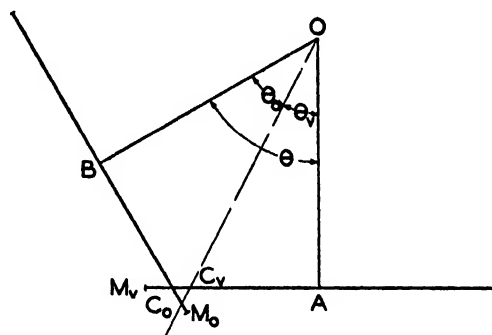


FIG. 7. The geometrical relation between the cameras.

from the fiducial distance to give a corrected value of the distance AC_v . Although M_vC_v is measured on film it is always short (2.8 cm. maximum), so the shrink correction is assumed to be negligible. This assumption was checked in several cases by measuring the apparent fiducial distance on the film and multiplying M_vC_v by the ratio of this apparent fiducial distance to the true fiducial distance. In no case had the assumption introduced an error greater than 3/10 min.

All measurements were made with a comparator that reads directly to 0.002 mm. For objects not at infinity, correction to the computed angle was made to allow for non-coincidence of the camera lenses.

On pictures taken directly over the city where much sharp detail was available several pairs of conjugate points were identified and the interlocking angle calculated for each pair. The results for several photographs are shown in Fig. 8. The abscissa is the distance of the image point from the fiducial mark of one of the cameras. These graphs indicate that even after allowance has been made for over-all changes in the film large errors can occur owing to local distortions.

The cause of this local film distortion has not been investigated, but it has been suggested that it may be caused by water drops drying very quickly and shifting the emulsion.

Although the errors found in local film shrink were large enough to account for the discrepancy between the calibrated and apparent angles of the mount, it was felt that a positive check on the calibration was desirable. Hence the mount was set up in the laboratory and pictures were taken on both plates and films. The graph of Fig. 9 shows the angle obtained on plates and on two

consecutive pictures taken on the rolls of films. The results from plates check very well with the calibrated angle of $60^{\circ} 30.5'$, but although the film was processed carefully by hand, some discrepancy is still indicated, especially in the first picture.

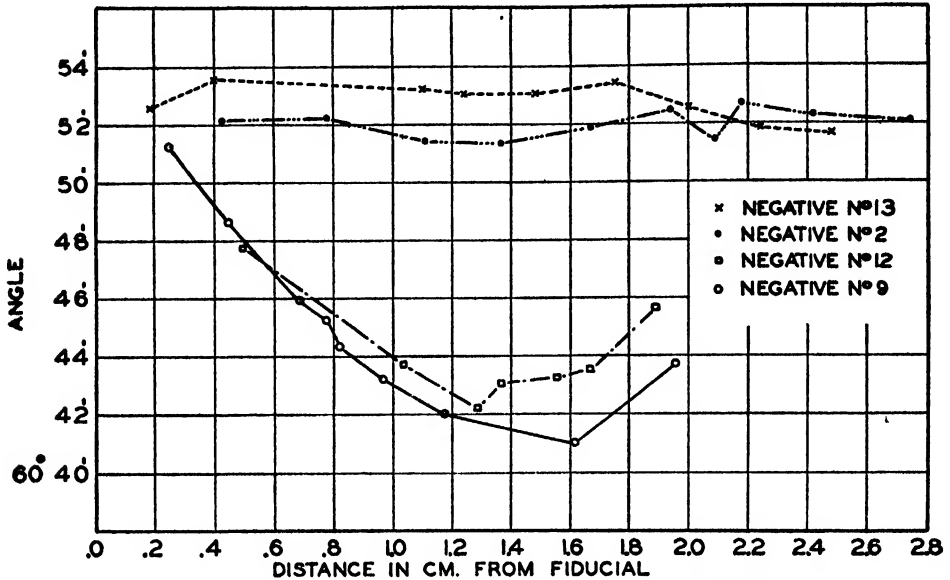


FIG. 8. Variation in the calculated interlocking angle due to local film distortion. The angle is between the vertical and the right oblique cameras.

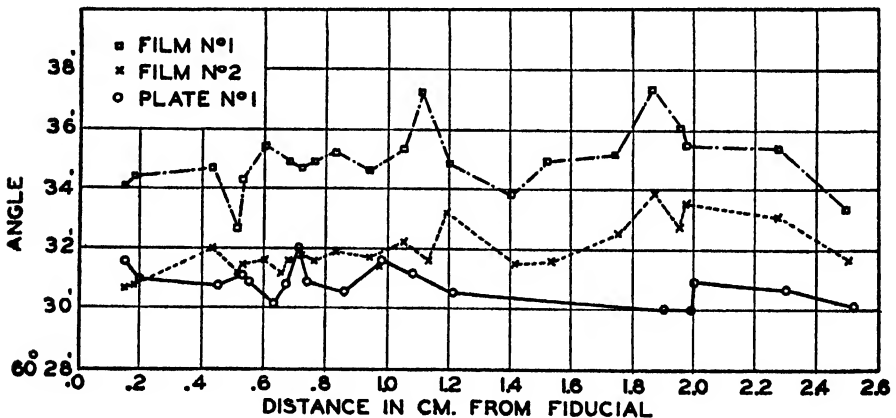


FIG. 9. Comparison of photographs on plate and film. The angle is between the right oblique and vertical cameras. This angle was calibrated at $60^{\circ} 30.5'$.

The sudden small changes on all of these graphs could be repeated but it is felt that this is due to the fact that the precision of measuring is greater than the accuracy of identifying conjugate points and that they are therefore not significant.

The effect of temperature rise due to heat from the illuminating lamp is an interesting illustration of the care necessary in making accurate measurements of photographic film. This effect is illustrated in the graph of Fig. 10. In

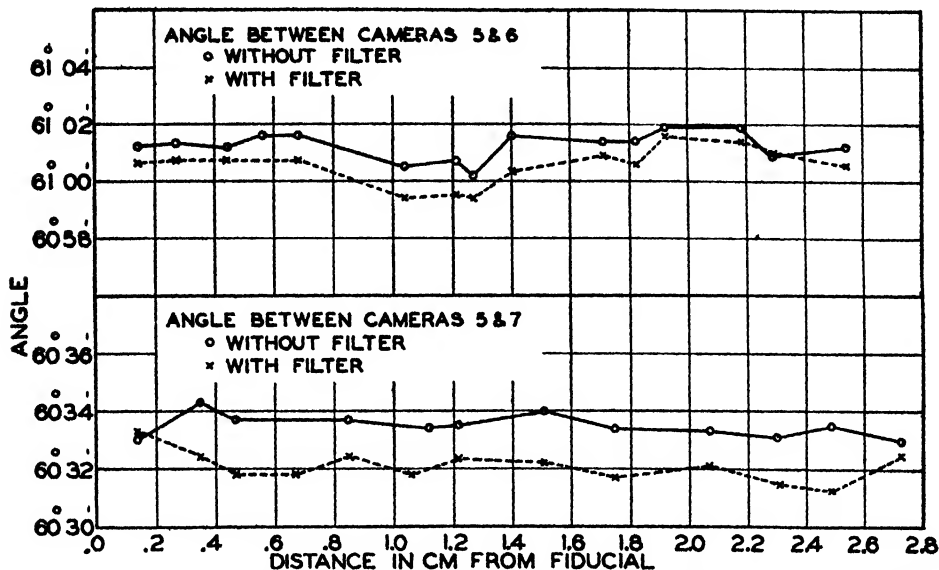


FIG. 10. Temperature effect on film. Camera No. 5 is vertical, No. 6 right, and No. 7 left.

the first curve, readings were taken with illumination falling directly on the film; in the second, heat absorbing filters were mounted between the lamp and the film. The change in angle is quite definite although the rise in temperature due to the lamp was not more than 15° F.

Conclusion

The tri-camera mount is mechanically rigid, but the lack of synchronization of the shutters may lead to errors as high as three minutes. The greatest source of error in using tri-metrogon methods is in local film distortion, which can lead to errors of 12 min. When paper prints are used this error will probably be greatly increased.

Acknowledgments

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The authors wish to express their appreciation to Dr. L. E. Howlett for his advice and assistance in this project.

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DIMENSIONAL CHANGES IN SAFETY TOPOGRAPHIC AERO FILM UNDER SERVICE CONDITIONS¹

BY P. D. CARMAN²

Abstract

A study has been made of the dimensional changes in Eastman Super XX Aero Topographic Safety film exposed in routine survey operations. Scale error varies from $\pm 0.02\%$ to $\pm 0.45\%$, distortion from -0.11% to $+0.08\%$. The largest errors are due to variations in the temperature and humidity to which the film is subjected. Control of these is recommended. Smaller errors indicate need for improvements in film base and need for a better film squeegee before the drier.

Introduction

Papers dealing with dimensional changes occurring in photographic film have been published by Calhoun (1), Clark (3), Davis and Stovall (4), Eastman Kodak (5), and Tupper and Clark (7, pp. 208-225). Only that by Calhoun gives information on the behaviour of film in normal use as well as under laboratory conditions. Unfortunately it deals only with motion picture film, not survey film. Some unpublished data on aerial film have been made available to the author (2).^{*} These data again deal with dimensional changes measured under laboratory conditions.

It is the purpose of the present study to provide general information on the dimensional changes likely to be encountered in survey operation, with a view to determining what accuracy is now available to the photogrammetrist and to ascertaining what improvement is possible by acceptable revisions of service procedure. In this study, the only departure from routine conditions is the measuring of the film under constant humidity and constant temperature. This is necessary to any attempt at interpretation of the changes. The further errors which would have arisen from random ambient conditions can be estimated readily.

Experimental Procedure

Measurements were made on films that had been exposed in regular R.C.A.F. tri-camera survey operations. The K-17B cameras used had previously been modified by the National Research Laboratories to make them suitable for accurate survey photography (6). One of the modifications had been the provision of fiducial marks attached to the camera body. These fiducial marks were designed so that the distances between certain edges of opposite pairs would provide accurate reference dimensions. To obtain these fiducial distances initially a photographic plate placed in the film plane of the

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^{*} And secret reports of the British Ministry of Aircraft Production.

camera was exposed to light coming through the camera lens. The distances between the fiducial mark images so produced were then measured. All were approximately 8 in. (200 mm.).

Six rolls of film were studied. They consisted of two groups, T49 and T50, each containing the concurrently exposed rolls, *L*, *C*, and *R* (left, centre, and right). Measurements of the fiducial distances were made on groups of four negatives at each of four positions in the roll. These positions were at the beginning of the roll, at the end of the roll, and two intermediate positions. One of the two intermediate positions was chosen to fall at the beginning of a flight line. The other was remote from the ends of any flight line. In addition, a set of measurements was made at frequent intervals throughout the entire length of one roll. Results of the first series of measurements are given in Fig. 1. Results of the second series of measurements are given in

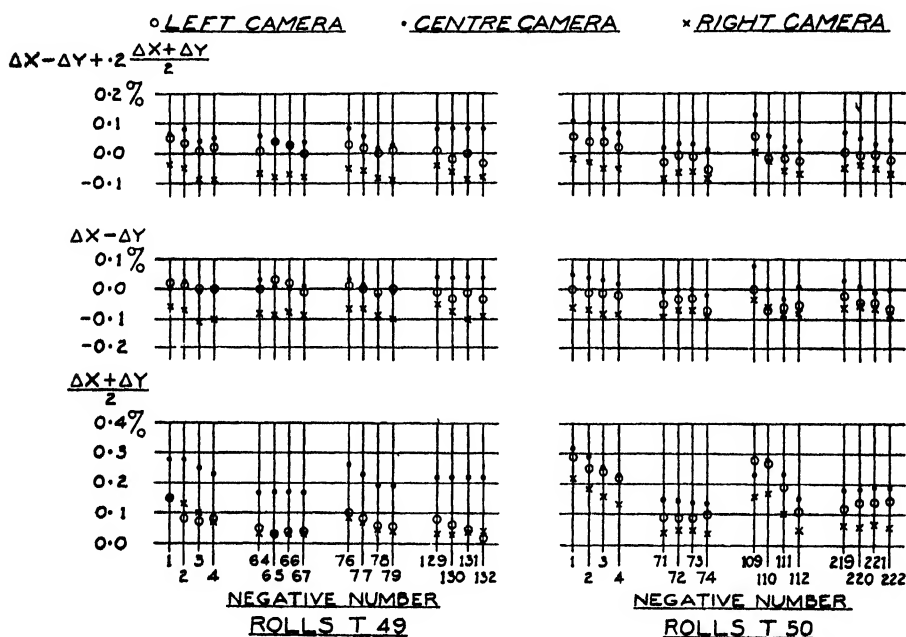


FIG. 1. Scale change and distortion on selected negatives from six rolls.

Fig. 2. The *X* direction has been taken parallel to the length of the film, the *Y* direction is at right angles to it. A positive value of ΔX or ΔY indicates enlargement or 'stretch' of the film between exposure and measurement.

Accuracy

The films were measured at 64% relative humidity and 70° F. For the first type of measurement, each group of four negatives was left unrolled under these conditions for 48 hr. To ascertain the accuracy being obtained, a considerable number of measurements were completely repeated after intervals

of up to three weeks. The maximum spread of values obtained was 0.02%. Most discrepancies were 0.01% or less. Hence the values given are correct to within $\pm 0.01\%$.

For the second type of measurement it was impracticable for each negative to be conditioned unrolled. However, at the time these measurements were made the roll had been in the constant humidity, constant temperature room for about two months and had been wound through several times. There are no indications that the resulting accuracy is significantly worse than that obtained in the other type of measurement.

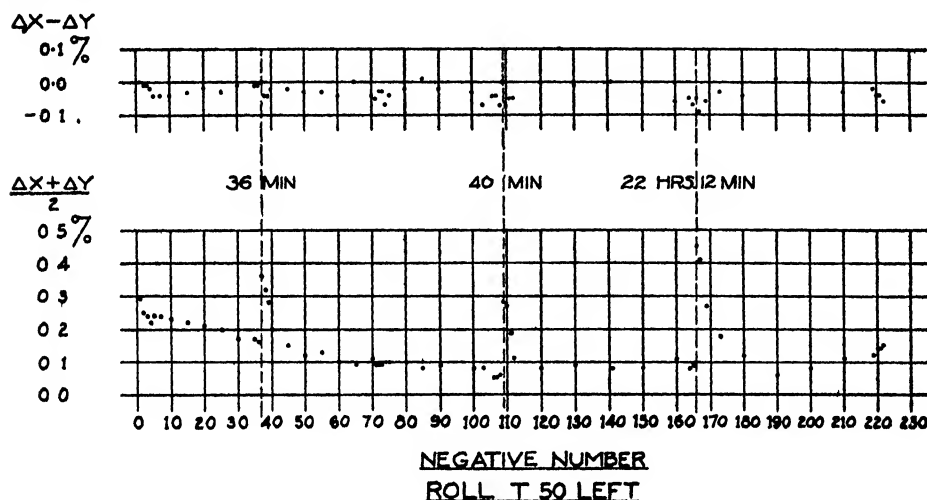


FIG. 2 Scale change and distortion throughout one roll. Broken lines indicate beginning of flight lines after intervals shown.

Related Data

Certain subsidiary data are required to permit an analysis of the results obtained.

Owing to the fact that the films were exposed under service conditions, only data regularly recorded are available. The relevant portion is given in Table I.

To obtain information on the film temperatures to be expected, a flight was made with thermocouples installed inside the magazines. At 20,000 ft. with an outside air temperature of -15°C . and with no heat turned on in the camera location, magazine temperatures were: left, 14°C .; centre, 9°C .; right, 14°C . The 'greenhouse' effect is very pronounced at the camera position in the nose of a B25 aircraft. The centre camera is lower than the obliques and shaded by the mount.

It is also necessary to have certain approximate data on the physical properties of the film—Eastman Kodak Topographic Safety. Measurements of the coefficient of linear expansion with temperature obtained by an English worker* are given in Table II.

* Secret reports of the British Ministry of Aircraft Production.

TABLE I
EXPOSURE DATA

Roll	Emulsion	Neg No	Time photos taken	Date 1945	Altitude ft	Magazines	Outside air temp at altitude, °C	Outside air temp. at take-off, °C.	Take-off time
T49	55-343-3	1-46	1045-1106	13 April	19 900	A	-26	+ 5	0916
		47-75	1010-1024	16 April	19 672	A	-19	+ 6	0900
		76-103	1042-1059	16 April	19,672	A	-19	+ 6	0900
		104-132	1027-1041	1 May	19,025	A	-12	+10	0930
T50	55-343-8	1-36	1110-1124	1 May	19 025	C	-12	+10	0930
		37-108	1200-1225	1 May	19 025	C	-12	+10	0930
		109-165	1305-1330	1 May	19,025	C	-12	+10	0930
		166-222	1142-1204	2 May	18,844	C	- 9	+12	—

TABLE II

Type of film	Coefficient of linear thermal expansion	
	X, along hlm	Y, across film
American type 1A, Class L, topographic base	72×10^{-6}	104×10^{-6}
Kodak topographic acetate base made in America and coated at Harrow, England	61×10^{-6}	71×10^{-6}

Coefficients are for a relative humidity of 75% and are per degree centigrade.

Adequate information on change of length with change of humidity for this type of film could not be found in the literature. Hence sufficient work was done to obtain an indication of this effect. Film conditioned at 64% relative humidity and 70° F. was transferred to a desiccated chamber at the same temperature. Measurements of shrink were made over a long period following the transfer. Results are presented in Fig. 3. The film used was Eastman Kodak Topographic Safety but it was not from the survey rolls studied.

Discussion

Scale error $\left\{ \frac{\Delta X + \Delta Y}{2} \right\}$ ranges from + 0.02% to + 0.45%

Distortion $(\Delta X - \Delta Y)$ ranges from - 0.11% to + 0.08%

Scale Error

With regard to scale error, a prominent effect is the transient increase at the beginning of each flight line (see Fig. 2). This can be attributed to the fact that these negatives had time before exposure to become conditioned to the humidity and temperature existing in the cameras. The minimum interval

between lines is 18 min. During this interval the position of the film in a magazine is as follows. The negative next to be exposed is in position in the focal plane. The one to follow it is partly (4 in.) in transit over the feed guide and roller, partly ($4\frac{1}{2}$ in.) on the outside of the supply spool. The

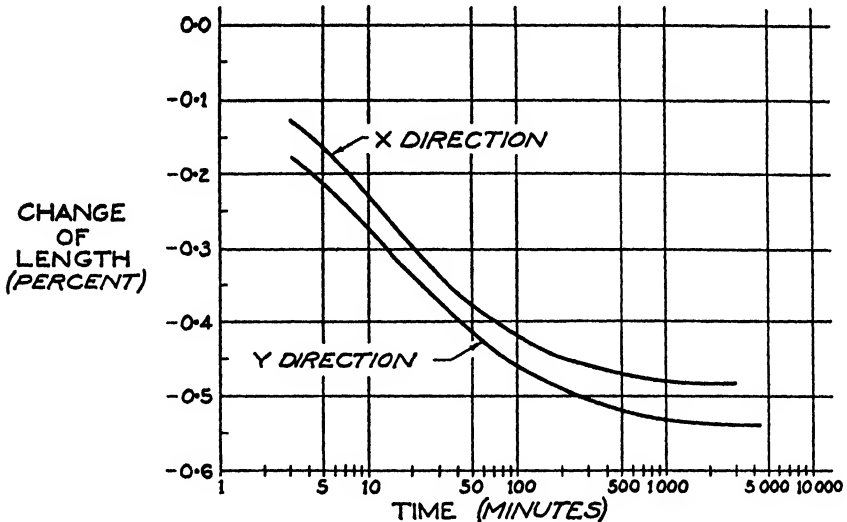


FIG. 3 Conditioning shrink on transfer from 64% relative humidity, 70° F, to a desiccated chamber at the same temperature.

third one is partly ($7\frac{1}{8}$ in.) on the outside of the supply spool and partly ($1\frac{3}{8}$ in.) on the second layer. The fourth one is entirely on the second layer of the supply spool. (Dimensions are for supply spool half-full.) The observed scale change decreases in magnitude in the above order, which is the order of degree of exposure to conditioning. (It might appear that the second negative is exposed equally to the first. However, the second is inside the magazine where the dryness of the air will be affected by the mass of film present.)

Fig. 2 clearly shows the gradual increase in scale change that occurs toward the beginning of each roll. This increase may be attributed to the gradual conditioning of the entire roll from the outside inward. The initial peak is missing here because a few test exposures were made shortly before the beginning of the flight line.

It is interesting to note that the peak at T50L 166, which is much higher than the other two, corresponds to a much longer conditioning line.

The conditioning could be to low humidity or low temperature. It is indicated that the film temperature would be between 0° and 20° C. as compared with the 21° C. at measurement. For an average coefficient of 77×10^{-6} , 20° C. would produce a scale change of only 0.15%. This maximum value is insufficient to account for the observed changes.

The temperature difference between the outside air and the camera compartment would result in a very low relative humidity. (For an outside humidity of 50%, an outside temperature of $-15^{\circ}\text{C}.$, and an inside temperature of $12^{\circ}\text{C}.$, the inside humidity would be 6.6%.) Hence it can be seen from the data of Fig. 3 that this alone could account for almost the entire scale change.

The two effects combined account for the observed changes.

A few further points in connection with a scale change are worthy of note. There is a suggestion of an increase in scale change toward the end of the T50 rolls. This could be attributed solely to temperature conditioning working out from the metal core. Humidity conditioning might also contribute to the effect. It would not be present in the T49 rolls since they were not used to the end. The generally positive value of scale change can be attributed to two causes. The measuring humidity is somewhat higher than the humidity in which the film was spooled by the manufacturer (by about 4%, corresponding to a scale change of 0.03%). A slight conditioning takes place before exposure even with the 30 sec. picture interval.

Permanent shrink has been neglected in the above discussion. It is known to be small during processing, and is probably not large for topographic base even over the 10 month interval between exposure and measurement. Correction for any present would tend to shift the scale change plots upwards somewhat without invalidation of any of the explanation.

Differences between negatives exposed simultaneously in different cameras are not clearly explicable. Possible causes are discussed later.

Distortion

Distortion effects cannot be accounted for as satisfactorily as scale changes. One would expect ΔY to be 10 to 40% larger than ΔX (1)*. Thus $\Delta X - \Delta Y$ would be equal to $\frac{\Delta X + \Delta Y}{2}$ times a constant between -0.10 and -0.33 . From Fig. 1, the average scale change is 0.139% and the average distortion -0.028% . These figures bear a ratio of 1 to -0.20 , which is in the above range.

However the ratio varies widely with individual negatives, from -3.0 to $+1.0$. A plot of this ratio would tend to be misleading since large changes in ratio arise from small changes in distortion on negatives for which the scale error is small. Instead the top graph of Fig. 1 is a plot of the measured distortion minus the distortion that would have occurred if the ratio -0.20 had been uniformly effective. This graph demonstrates that the average value of the distortion is without much significance since correction for it makes little difference in the distortion range.

Probable Causes of Remaining Errors

Because the observed errors result from conditions that at present are neither controlled nor measured in service no rigorous explanations can be

*And secret reports of the British Ministry of Aircraft Production.

established for some of them. This is not completely satisfactory from an academic point of view, but is of little practical significance compared to the current value to photogrammetry of the information obtained in the test. Although rigorous explanations are not always possible, plausible ones can be advanced for the remaining errors.

Most apparent of these errors are the persistent differences between rolls that appear both on scale error and distortion (Fig. 1). For each negative number the value for the centre camera is distinctly greater than that for the right camera, with average differences of 0.10 and 0.08% on distortion and 0.16 and 0.11% on scale change for T49 and T50 respectively. Values for the left camera are more random. They usually lie somewhere between the other two. Yet for T49, left camera values are close to those of the right camera on scale error, and close to those of the centre camera on distortion, while, for T50, left camera values are generally intermediate.

This effect could be due either to variation in exposure conditions or to variations in the properties of the film. The latter explanation requires a somewhat fortuitous repetition in the loading of the centre and right magazines. The chances of such a repetition are not unduly low and might have been increased by systematic film handling. This explanation would account for the differences between T49 left and T50 left. Properties of the film that might vary to produce the effect are coefficients of linear expansion with temperature, dimensional changes due to processing, and dimensional changes during storage. One case of variation in temperature coefficient has been found by an English worker while investigating a different aspect of film behaviour (see Table II). For exposure at 0° C. variations of the size he found would account for almost half the average difference between films exposed in the centre and the right cameras.

Variation in exposure conditions seems unlikely to have caused the effect. This is seen from the following consideration of the various possible causes.

1. The centre camera, which is indicated to be the coldest, shows the biggest scale change. However, its lower temperature would produce an increase in relative humidity slightly more than sufficient to nullify the thermal contraction. There remains the remote possibility that the relative humidity was so low as not to be affected significantly by the temperature difference. Even admitting this, the observed temperature difference of 5° C. produces a scale change of only 0.04%, which is $\frac{1}{4}$ to $\frac{1}{5}$ the observed range. Thus there is little possibility of temperature variation causing the scale change differences and no apparent means whereby it could cause the distortion differences.
2. Relative humidity differences arising from ventilation variation are another possible cause of the scale error differences. However, the initial rate of conditioning—about 0.15% in three minutes—from Fig. 3 is not sufficient to explain the differences. Effective conditioning time could hardly be more than 90 sec. except at the beginning of a run. The humidity differences between cameras could not be great or the effects

of conditioning on the first few pictures of a line would differ markedly. Relative humidity, like temperature, cannot explain the distortion differences.

3. Errors in the positioning of the film relative to the fiducial marks are unlikely since the cameras were checked for this after modification. Clearance between the fiducial marks and the film was nominally 0.003 in. All clearances were kept under 0.005 in.
4. Overexposure of the fiducial mark images in the sky portion of the obliques might contribute toward the persistent distortion differences but would not be sufficient to provide for the approximately 0.2 mm. (0.1%) required.

To demonstrate this, a 1 mm. slit was contact printed on Aero Super XX film with a wide range of exposures. Development was a laboratory duplication of that used in service. For resulting densities between 0.8 and 1.6 the variation in image width was less than 0.005 mm. Even for a density range from 0.4 to 3.0 the variation in image width was only 0.060 mm.

5. Mechanical tension on the film may also be considered. The greatest tension would occur when the film in the focal plane shrank from conditioning effects while the pressure back was down and the suction on. To duplicate the suction conditions which were not accurately known, tests were made in an aircraft in flight. It was found that a force of 30 lb. was required to pull film through the magazine with the back down and suction on. (The suction, measured in the line at a point 6 ft. from the magazine, was $1\frac{1}{2}$ in. of mercury below the surrounding atmospheric pressure.) Hence the forces arising due to contraction of the film would produce an elastic stretch of only 0.03% (2).

Thus there seems little likelihood that exposure conditions alone can have produced the differences between rolls exposed simultaneously. Hence it seems probable that these differences are due primarily to variations in the properties of the film.

Random Errors

Allowing for the various forms of persistent errors that have been discussed, it is apparent that random errors remain. The maximum values of these are of the order of $\pm 0.03\%$ on scale and $\pm 0.05\%$ on distortion. (As is to be expected statistically, the latter is about twice the former.) These random errors are of the same order as those found by other workers in the measurement of distortion arising in processing. Hence there is no reason to attribute them to any other cause.

It should be noted that there is no evidence for treating these random errors as percentage errors. They are more likely to be linear displacements independent of the distance over which the measurements are made (6). Small water drops on the film as it enters the drier are a probable cause.

Conclusions and Recommendations

Scale error $\left\{ \frac{\Delta X + \Delta Y}{2} \right\}$ ranges from + 0.02% to + 0.45%.

Distortion $(\Delta X - \Delta Y)$ ranges from - 0.11% to + 0.08%.

Scale change is not an extremely serious difficulty to photogrammetrists, provided it is known, since corrections can be applied. However, if it is not determined for every negative, errors up to 0.4% may arise since scale varies considerably throughout a roll. This variation can be reduced to less than 0.1% by maintaining the film at spooling temperature and humidity prior to and during exposure. Such a procedure is recommended. Since its introduction will take considerable time the following three interim measures are suggested. (1) Obtaining the film from the manufacturer in humidity sealed tins would provide easy maintenance of humidity conditioning up to the moment of loading. (2) The cameras should be maintained at 70° F. to avoid temperature effects. (3) The cameras should be started several exposures before the beginning of each line so that the first few negatives may be discarded. For intervals of about half an hour between lines, five such preliminary exposures are sufficient. For a 24 hr. interval the number should be about 15.

For the above measures to be fully effective, the film should be reconditioned to spooling temperature and humidity for printing.

Distortion is much more serious to the photogrammetrist than scale change since he has no convenient method of correcting for it. The measures recommended for reducing scale error may also reduce distortion somewhat, but the distortion problem is largely one for the film manufacturer.

Random errors cause a total variation of 0.06% on scale, 0.10% on distortion. To reduce these it is recommended that a highly efficient squeegee be installed on the processing machine between the washing tank and the drier.

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